PLANT WATER RELATIONS IN A MODEL AGROFORESTRY SYSTEM

by

MOHAMMED MOHIUDDIN
BSc (Hons) MSc (Botany)

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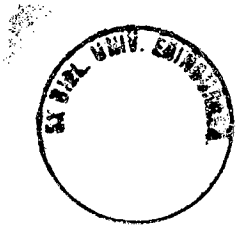

MEMORIAL

In memory of my father
Mohammed Bedar Hossain Chowdhury
Who died on August 14, 1989
Two weeks before I left my country
For this study

DEDICATION

To my mother
Mosammet Mahfuza Begum
With love and respect

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ABSTRACT

This study reports the results of experiments on growth and physiology of an agricultural annual (French bean) and a young tree (poplar) in relation to limited soil water. Competition and complementarity between the species were evaluated in a model agroforestry experiment. The experiments were carried out in a greenhouse and growth cabinet with plants grown in pots containing sandy-loam compost. The species studied were *Phaseolus vulgaris* cv. Argus and *Populus trichocarpa* X *P. deltoides* cv. Raspalje. The main aim of the study was to characterise the responses of both species to different soil water supply regimes, shoot water supply by roots and chemical signalling from the roots in drying soil and to show how these responses could be used in the selection of suitable agroforestry species for dry regions.

Bean and poplar plants showed differential growth responses although both the species performed better in mixed stands than in monoculture when soil water was severely limited. Bean plants were more competitive than poplar, although both species showed complementarity in exploration for soil water. As the soil dried leaf water potential declined. Stomatal conductance of both species was more closely related to pre-dawn leaf water potential than to mid-day leaf water potential, indicating the importance of soil water status.

Experiments with both vertically and horizontally divided root systems showed that bean and poplar plants with at least half of their root system in moist soil were able to maintain leaf water status as well as plants with all their root systems in moist soil. Furthermore, stomatal conductance and leaf expansion of both species were affected directly by soil drying independent of leaf water potential. Abscisic acid (ABA) synthesized in the roots of both species in drying soil appeared to be transported to the leaves in the transpiration stream where ABA regulated stomatal conductance and leaf expansion. The increased ABA in xylem sap as an immediate result of soil drying induced stomatal closure *before* any increase in bulk leaf ABA. Increased concentration of ABA in xylem sap subsequently contributed to the substantial rise in bulk leaf ABA. Bean plants were found to be more *sensitive* to soil drying than poplar with respect to stomatal closure and the reduction in leaf expansion. The ABA concentration in the xylem sap of unwatered bean plants necessary for initial stomatal closure was higher than in poplar when expressed in relation to the ABA concentration in well-watered plants.

Complementarity between species, in their physiological responses to water stress is important to establish in developing agroforestry systems for dry regions.

CONTENTS

CHAPTER 1

Tree-Crop Interaction in Agroforestry and Aims of the Study

1.1 Introduction

Ever increasing population, land scarcity and lack of proper land use systems are common in the world, particularly in the developing countries. So shortages of food, fuel and timber are inevitable. On the other hand, much fragile crop land and forest area, as well as marginal land could be better utilized. With a view to improving the situation, all available land, including fragile crop and forest land, as well as all marginal land, should be taken into a scientific land management system, in which both annual and tree crops could be grown simultaneously or sequentially, namely agroforestry. By the implementation of this system, sustained productivity of a particular area of land may be possible.

In agroforestry, ecological interactions between trees and crops are common involving the utilization of above and below-ground environmental resources of light, water and nutrients. The interactions might be complementary (e.g. Huxley, 1983; Wiersum, 1984; Young, 1986) or competitive (e.g. Iwaki, 1959; Hall, 1974a &1974b; Trenbath and Angus, 1975; Leyton, 1983; Campbell, 1989). The concepts of competition and complementarity among plants for environmental resources are important to the goals of agroforestry.

Availability of soil water is uncertain, particularly in monsoon and savanna climates (Monteith, 1977). Because of periodic drought, shortage of soil water is common in Bangladesh, particularly in the northern part of the country and is increasing at an alarming rate. In this circumstance, the component species in an agroforestry system may compete with each other for soil water to an extent that may lead to a negative interaction between the components (Ong *et al.*, 1991). However, possible differential responses of trees and field crops to drying soil could overcome this situation.

The responses of plants to soil drying are not always quantitatively similar, although similar growth and physiological processes are affected. Trees and field crops

growing together in a water shortage area may modify soil drying. The process of soil drying may also modify plant responses to dry soil. In recent years attention has focussed on the role of roots in dry soil, rather than on shoot water status, in influencing growth and stomatal regulation. Therefore, a study on the responses of annual plants and young trees to soil drying in a model system could resonably provide a basis for selecting suitable species for successful agroforestry in a water shortage area.

1.2 Prospects for agroforestry

As we know, "Agroforestry has been defined as a sustainable land management system which increases the overall yield of the land, combines the production of crops (including tree crops) and forest plants and/or animals simultaneously or sequentially, on the same unit of land and applies management practices that are compatible with the cultural practices of the local population" (King and Chandler, 1978).

Since, land is a scarce commodity in most of the developing countries, the carrying capacity of the land is already overstretched. Out of 117 developing countries, 54 currently have insufficient land resources to meet their people's demand for food (Higgins *et al.*, 1983). With the consequent increasing demands for agricultural production, about 10 millon hectares of new land are also needed every year (Piemental and Hall, 1989). Deforestation, and the gap between deforestation and tree planting, is also another acute problem in the tropics. The security of a sustained supply of food, fuel, timber etc. from the available land resource might contribute to overcoming the situation to some extent. So the maintenance of sustained productivity of a particular land area through a scientific agroforestry management system is being given higher priority. Because of the scarcity of productive land, fragile crop lands and forests, generally unsuitable for modern agriculture, might none the less contribute to the process of sustained productivity through scientific land use practices. However, unscientific use of such land can cause a number of problems, notably soil erosion by which about 10-36% of rainfed cropland has been lost, leading to a predicted 29% reduction of crop production by the year 2000 (Higgins *et al.*, 1983).

Because of increasing reduction of tree cover (in addition to the use of fossil fuels), carbon dioxide concentration is increasing in the atmosphere leading to a predicted

increase in global temperature. Eventually, agricultural production may be adversely affected directly, so growing trees and agricultural crops in an agroforestry system may contribute to the improvement of environmental conditions, along with the alleviation of rural poverty. From the point of view of human need, agroforestry is a permanent land use system which can provide nutrients through fruit, vegetables, meat and eggs and also supply fuel wood, timber, ornamental and medicinal plants (Michon *et al.*, 1983). In addition, a typical agroforestry system involves symbiotic, economic and ecological interactions between woody perennials and non-woody annual crops with various sustainable forms of total land output.

Agroforestry systems have been practiced successfully in arid and semi-arid regions as well as in temperate and tropical regions. Although about 23% of land in Europe is under forest, Britain has only 9% forested land, so 90% of the national consumption of wood products has to be imported (see Alternative Enterprise for Agriculture in UK, 1986). To overcome this situation, alternative uses of agricultural land now include the introduction of trees on farmland. In the Indian subcontinent there is a long tradition of agroforestry systems, where trees are integrated extensively with crops and livestock (Singh, 1987).

With respect to Bangladesh, rural poverty and deforestation are critical and interrelated. Over-population and land scarcity accelerate the problem. Of the current 110 million population, more than 60 million are landless; 8000 ha of land are deforested every year and 35 - 45 % of forest land is either cultivated or encroached (Government of Bangladesh, 1987). Agroforestry systems are appropriate for resource-poor farmers, providing immediate returns from agricultural crops and long-term benefits from trees: homestead forestry provides over 80% of the national wood consumption (Douglas, 1982). So agroforestry has a large impact on allevation of rural poverty and on forest depletion as well. For the maintenance of soil productivity, planting trees in crop fields or along their boundaries may also be helpful. Recent agroforestry practices in different parts of the country, particularly in the northern part, are encouraging from the point of view of growth and yield (Bangladesh Agricultural Research Council, 1990). Thus in order to improve the existing agroforestry system and to test alternative systems, appropriate research is needed urgently.

1.3 Limitations of agroforestry

From an ecological point of view, competition between the components of an agroforestry system for space, sunlight, water and nutrients may take place. This may reduce growth and yield of the component species. Besides, because of excessive water loss from mixed cropping the shallow root system of tree species and the roots of crops may be susceptible to rapid soil drying and this can lead to the supply of chemical signals responsible for the reduction of stomatal conductance and growth (e.g. Blackman and Davies, 1985; Zhang *et al.*, 1987; Zhang and Davies, 1990*a*). The chemicals produced in drying roots may also be released into the soil with subsequent adverse effects on co-habiting species. Interactions may occur in other ways. There is a possibility that one component species may be a host to pests and insects, which may be harmful to the other. Sometimes, because of rapid regeneration of fast growing tree species, agricultural crops may be displaced and consequently the whole land area becomes covered with trees and agricultural production declines.

From the socio-economic point of view, because of competition between food and tree crops, the aggregate yield may be less than that of single crops in some situations. In the selection of appropriate species for a successful agroforestry system in a particular area, farmers may also face difficulty: inappropriate component species in any agroforestry system on a particular area may not grow well and consequently the total yield declines. Since agroforestry is a more complicated system for farmers, it is more difficult to manage than that of single crops, so farmers may resist growing trees together with agricultural crops.

1.4 Tree-crop interactions in agroforestry

Interactions between component species are inevitable in agroforestry and include ecological changes and depletion of environmental resources. Trees provide shelter to annual crops, resulting in a considerable reduction of evapo-transpiration from crop fields in some cases (Wiersum, 1984). In mixed stands of cacao and coconut a considerable (50%) reduction of evaporation has been observed relative to that of coconut grown alone, because of the reduction of air temperature (Nair and Balakrishnan, 1977). Trees also improve soil fertility through nitrogen fixation especially by some nitrogen-fixing trees (e.g. Acacia, Albizia, Erythrina and Gliricidia species, including *Leucaena leucocephala*), greater organic matter production and

recycling of nutrients (Young, 1986). Crop cover can also reduce soil evaporation loss, leading to increase in soil water status. On the other hand, mixed cropping may induce rapid soil drying because of excessive transpiration loss. The radiation interception by understorey crops may also be affected because of crowded overstorey canopy cover. Thus competition between component species may occur for above and below-ground resources. Light, temperature, wind speed and humidity are involved in above-ground interactions (Monteith *et al.*, 1991). Below-ground interactions include competition for water and nutrients.

Competition between plant species is an important phytosociological factor in plant succession which arises when the immediate supply of a single necessary variable falls below the combined demands of the component species (Clements *et al.*, 1929). Hall (1974a) defined competition between plants as the response of an individual plant or plant species to its total environment, as this is modified by the presence and / or growth of other individual or species. For instance, an optimum below-ground condition may also encourage competition between component species because of the promotion of growth of an understorey component (Campbell, 1989). In these circumstances, competition for light may also occur later as a result of the development of an overstorey canopy component. Since water, nutrients and light are the most common environmental variables in short supply, competition for these may often occur between component species in any agroforestry system with subsequent reduction of plant growth. If those environmental resources utilized for the growth and development of one component, become less available to the other component in mixed-cropping, yield loss often results (see Hewson and Roberts, 1973).

In an area of limited water availability the component species in agroforestry may compete with each other for soil water. Competition for nutrients generally depends on the nutrients in question and their availability. The rate of replenishment of nutrients, soil water relationships and nutrient uptake characteristics of the co-habiting species have significant impacts on this competition. Component species with similar rooting patterns may accelerate competition for soil water and nutrients. This generally occurs during the establishment of tree seedlings because of a restricted distribution of roots to the top soil layer by, for example, shallow rooted annual crops. Thus, because of competition for water in the top soil layer, the growth of tree seedlings may be affected, as reported by Muthana *et al.* (1985). The rooting pattern of shallow rooted annuals and deep rooted perennials is not always solely determined genetically but is much more affected by soil and climatic conditions (see Leyton,

1983). The roots of shallow rooted annual crops may often grow deeper under limited soil water (Sharp and Davies, 1985) to explore subsoil where they may also compete for water with perennials. However, although different plant species have different rooting pattern, maximum soil water depletion frequently occurs in the top soil layer (Eales, 1980). This may result from both superficial roots and surface evaporation, so that the root systems of component species in the upper soil profile may often be dehydrated. One hypothesis proposed is that such dehydrating roots could induce stomatal closure in the leaves before any decline in shoot water status is evident (e.g. Bates and Hall, 1981; Blackman and Davies, 1985; Gollan *et al.*, 1986), and this may indeed be a plant's earliest measure of soil water status. It follows that the component species of an agroforestry system that utilize water in the subsoil profile and avoid any competition, may be able to sustain productivity in a periodic water shortage area.

Without a doubt, component species with different rooting patterns may extract water and nutrients from different parts of the soil profile and thus reduce competition. As a result, more water and nutrients may be extracted by a mixed cropping system than by individual crops, leading to an increase in total biomass production (Huxley, 1983). Moreover, some deep-rooted species transfer water from deeper to shallower soil layers, resulting in a higher content of soil moisture in the topmost soil layer (Mathavan *et al.*, 1985) that is more available to the shallow rooted component (Corak, *et al.*, 1987). Component species differing in ability to utilize nutrients in different forms may also apparently reduce competition for nutrients (King, 1979). However, if component species have similar root distributions and competitive ability, they may share the resource equally until it is exhausted and then suffer simultaneously from the effect of depletion of the resource. Thus component species with differing competitive ability at any one time may minimize competition (King, 1979). So, the selection of components of agroforestry systems and their management must account for these issues.

Competition for light between component species depends on their canopy structure and is an important aspect of above ground interactions in mixed cropping. The understorey canopy is likely to have a reduced rate of photosynthesis because of the lower irradiances experienced. Since dry matter production is linearly related to intercepted radiant energy (Monteith, 1977), yield is affected. For example, the growth of green grams was suppressed because of shading from buck wheat when grown together (Iwaki, 1959). In shaded sugar beet, decreased interception of

incoming radiation is the main cause of lower net assimilation rate and leaf area, with subsequent reduction of dry matter production (Watson *et al.*, 1972). In order to overcome this negative interaction, species of different leaf area duration can be grown so as to intercept light at different times. In mixed cropping of fast growing sorghum and slow growing pigeon-pea such sharing of light can occur (Ong *et al.*,1991). In other words, component species should be selected with phenology, canopy architecture, growth pattern and rates of growth that permit maximum light interception by both over their growing seasons.

Manipulation of competition by intercropping has been exploited by farmers in many parts of the world. It is an important feature of low input agriculture in the tropics where sunlight is abundant, rainfall is seasonal but unreliable and nutrients are deficient. For agroforestry to be successful, the tree and field crops should be compatible and complement each other in growth pattern over most stages of their lives. However, the competitive ability of any crop component depends on its capacity to adapt to environmental stresses as well as upon the behaviour of co-habiting species.

1.5 Statement of the problems and the aims of the study

Shortage of soil water and periodic drought are common in several parts of the world as a result of uncertainity and seasonal variation of rainfall. Because of considerable monthly variation of rainfall in Bangladesh (Bangladesh Meteorological Department, 1987), the tree planting season (July-October) is followed by a period of drought. As a result, about 20 to 50 percent of tree seedlings planted every year die during the following dry months (see Hassan, 1987). He has proposed that working the soil in the top 20-30 cm may possibly encourage tree seedlings to spread roots downwards below the lower limit of soil-moisture-extraction before the onset of the dry season. In these circumstances, planting annual crops between the tree seedlings is economically logical and could improve the use of below-ground resources, with possible extra economic return from the annual crops. Transfer of this knowledge to farmers for their homestead agroforestry system may encourage the introduction of trees onto agricultural cropland.

Although many workers have reported the influence of soil water on plant growth and physiology, there are few studies on mixed cropping of annuals and woody

perennials, particularly during the establishment of tree seedlings. Growing annuals and tree seedlings together on a particular area of limited soil water may cause rapid drying of the top soil layer because of higher transpiration from the mixture than from either species grown alone, with subsequent partial root drying of both species. The influence of partial root drying in dry soil on plant growth has not yet been investigated sufficiently. In contrast to the conventional view of stomatal closure in response to decline of leaf water and turgor potential (Turner, 1974), the stomatal conductance of plants rooting in drying soil is not always associated with leaf water status (Bates and Hall, 1981; Turner *et al.*,1985). Although the general pattern of responses to water stress is similar in many plants, there are important differences in the responses of stomatal (Ludlow, 1980) and developmental (Angus and Moncur, 1977) processes. So the aim of the project is **"To investigate the responses of an annual and a young perennial tree growing in monoculture and in mixed stands in relation to soil water supply"** to answer the following questions with the aim of obtaining a better understanding of the physiological and growth processes leading to a better utilization of land:

* Do annual crops compete with or complement the growth of tree seedlings during soil drying?
* Do young trees in their early stages of growth compete with annual crops when soil water supply is limiting?
* Do component species in mixed stands accelerate the drying of the top soil layer under limited soil water supply?
* Do roots in drying soil have an influence on growth and physiology of shoots?

As we know, not all trees and annuals grown in monoculture and in mixed stands perform the same in relation to soil water supply. The present study on dwarf Franch bean (*Phaseolus vulgaris* L. cv. Argus) and poplar (*Populus trichocarpa* X *P. deltoides* cv. Raspalje) will lead to the development of a method for selecting suitable component species for successful agroforestry cultivation in a particular area. With a view to fulfilling the aims of the project, experiments have been conducted with the following specific objectives:

(a) to examine the responses of bean and poplar when grown in mixed stands compared to that in monoculture with various soil water regimes;

(b) to determine the growth and physiological response to soil drying in relation to

shoot water supply;

(c) to determine the influence of partial root drying on growth and stomatal conductance in relation to chemical signals from the roots; and

(d) to assess the change in xylem sap ABA concentration and its influence on growth and stomatal conductance during soil drying.

1.6 Comments on species

The poplars are a large and diverse group of woody perennial plants belonging to the genus *Populus* that comprises about 30 species. Most of the species are native to large areas of Europe and North America, with some from China. Poplars have become an important part of forestry in many parts of the world because of their excellent growth on a wide range of sites (see Ceulemans, 1990). Moreover, poplars have proved to be better suited for short rotation intensive culture (SRIC) biomass production than some other genera. Agroforestry with poplars is well developed in a number of countries such as Italy, New Zealand and Australia. In New Zealand, agroforestry poplar plantations play a significant role in the control of soil erosion. Plantations poplar have also been a successful agroforestry system in Australia providing shelter which gives a better microclimate for pasture growth and makes cattle more comfortable and productive. The poplar used in this study is a hybrid of *Populus trichocarpa* X *P. deltoides*, cv. Raspalje.

Generally poplar is propagated vegetatively and planted as cuttings (without roots), sets (rooted cuttings) or barbatelles and thus is suitable for the maintenance of genetic purity, resulting in plantations of trees with identical parents. Economically, the wood of poplar hybrids is used to make furniture and for pulpwood and plywood, as well as for making matches. The leaves are highly nutritious as fodder for cattle. Evergreen clones provide fodder almost year round. Pruning of poplar plants, essential to improve tree shape for good timber, can provide both cattle fodder and fuel wood.

The French bean (*Phaseolus vulgaris*) comprises a number of varieties, originating from South America and is usually grown in areas of medium rainfall from the tropics to the temperate region. In the region of arid sub-tropics French beans are also grown under irrigation. French beans are usually propagated from seeds and grown

successfully in light sandy to heavy clay soil as well as in peaty soil. French bean is usually interplanted with other crops like maize, sweet potato, cotton and coffee in tropical Africa. Intercropping of French bean with *Leucaena leucocephala* gives greater tree biomass production (Maghembe *et al.*, 1986) and intercropping with *Eucalyptus camaldulensis* results in significantly higher growth of the tree seedlings (Chingaipe, 1985).The variety of *Phaseolus vulgaris* used in this study is Argus.

French beans are widely cultivated in some parts of the tropics for their immature edible pods, dry ripe seeds, green-shelled and leaves as pot-herbs. Green, immature pods are also popular in Europe and United States as vegetables. Dried straw is also used as forage.

CHAPTER 2

Plant water relations: a review of literature

2.1 Development of water stress in plants

The movement of water in the soil-plant-atmosphere continuum depends on the thermodynamic concept of a gradient of water potential across resistances in the pathway. As a result, differences in water potential provide the driving force for water transport in plants. Transpiration makes this gradient inevitable because transpiration dehydrates leaves resulting in a lower water potential in the leaves than in the roots (Westgate and Boyer, 1984). The lower water potential in leaves provides a driving force for water movement out of adjacent organs, resulting in subsequent loss of water from the stem followed by the roots. Such a decline of water potential can lead to the development of water stress. Water stress refers to the effect on processes of the decline in the components of total water potential, such as the turgor and osmotic potentials, which influence the growth rate and physiological performance of plants. However, the value of total water potential at which the turgor potential appreciably declines varies from species to species and may also vary with the conditions to which the plant has been exposed previously.

Water stress develops in leaves because of the considerable frictional resistances to flow in the water pathway and may occur on sunny mornings, even in plants growing in well-watered soil, since during this period water is readily available for transpiration in the parenchyma cells of turgid leaves (Boyer, 1969). However, this water stress is temporary because of the reduction of transpiration in the afternoon. More severe, longer lasting water stress develops as removal of soil water in transpiration leads to lower soil water potential. When plants are growing in conditions of limited soil water larger hydraulic tensions are transmitted in the xylem column (Boyer, 1985) to the leaves because of the large resistance to water flow from the soil to the root surfaces (Passioura, 1982), resulting in a still larger decline of leaf water potential. Kramer (1988) asserted that shoot water stress usually develops before any significant stress occurs in the roots because of the dehydrating effect of the atmosphere. The reduction of leaf turgor despite turgor maintenance in roots of maize plants growing in drying soil (Sharp and Davies, 1979) supports these ideas. Boyer (1989) postulated that, as soil dries, the roots communicate the soil condition to

the shoot as a change in xylem water potential and by altering the rate of delivery of solutes to the shoot. Thus, the development of water stress in leaves is caused by the inevitable decline of leaf water potential as a result of transpiration.

On the other hand, during soil drying leaf and shoot functioning is not always related to shoot water potential, because plants may develop active control of shoot water status. Schulze and Hall (1982) pointed out that the same leaf water potential may be reached, either by strongly transpiring well-watered plants or by water-stressed plants with low transpiration rates. Moreover, it is possible for water stressed plants to exhibit higher shoot water potentials than well-watered plants (Jones, 1985), once the stomata have closed.

Evidence of a regulatory effect of soil drying on shoot functioning without any decline in shoot water status has been given by Blackman and Davies (1985); Gollan *et al.* (1986); Zhang *et al.* (1987). For example, Zhang and Davies (1989a &b) observed the dehydration of shallow roots even though deeper roots provided sufficient water to allow transpiration to occur at the potential rate. However, there are some reports suggesting that roots in dry soil may be at a higher water potential than the surrounding soil because roots in dry soil are hydrated by transport of water from roots in wetter regions (Mooney *et al.*, 1980; Sharp and Davies, 1985; Corak *et al*, 1987). At the present time, the relative importance of the root and the shoot as sensors of plant water stress during soil drying has yet to be fully evaluated.

2.2 Plant response to water stress

2.2.1 Expansion growth

Plants growth is directly related to rates of cell division and enlargement; both are reduced under water stress, but not to the same extent (Barlow *et al.*, 1980). McCree and Davies (1974) working with water-stressed sorghum found that both cell division and enlargement were equally important for leaf expansion. Generally, cell enlargement is more inhibited than cell division by water stress (Hsiao, 1973), as has been reported for maize (Acevedo *et al.*, 1971) and soybean (Bunce, 1977). Cell enlargement is one of the plant processes most sensitive to the development of water stress (Bradford and Hsiao, 1982), because cell growth is quantitatively related to cell turgor potential. Cell turgor is generally maintained by water entering cells down a

water potential gradient (Boyer, 1985) which results from cell wall relaxation. The movement of solutes to the cells may also help in the maintenance of turgor potential.

As soil dries, the soil water potential is reduced and the frictional resistance to flow increases resulting in decline in the shoot water potential. According to the conventional view, the effect of low water potential on growth has been attributed to the reduction of turgor (Boyer, 1968; Acevedo *et al.*, 1971, Sharp and Davies, 1979). Reduction of leaf growth has often been found to be associated with turgor decline in water-stressed plants (Takami *et al.*, 1982; Kramer, 1983). However, cell turgor can be maintained under soil drying when solutes accumulate in water-stressed cells (Hsiao *et al.*, 1976; Turner *et al.*, 1978) so that growth of plants can be maintained during water stress (Tyree and Jarvis, 1882). This osmotic adjustment (see Morgan, 1984) allows plants to maintain growth at low water potentials. Osmotic adjustment is an important adaptive mechanism allowing yield to be sustained under water shortage.

There are several reports of plant growth being affected by soil drying, even though turgor potential was maintained (e.g. Turner and Jones, 1980; Michelena and Boyer, 1982). This indicates that solute accumulation during soil drying may not fully compensate for the effect of limited water supply on cell expansion. Westgate and Boyer (1985) suggested that there is an interruption of sustained water supply to the growing cells because of decline in the water potential gradient between xylem and growing cells. This phenomenon was demonstrated by Boyer and Nonami (1990) in soybean plants growing in a medium with a reduced water supply. Since cell growth was inhibited, the accumulation of solutes in relatively small cells (Meyer and Boyer, 1981) failed to maintain the yield threshold. Hsiao *et al.* (1976) suggested that change in the threshold turgor potential with the development of water stress should be attributed to change in cell wall extensibility. Mathews *et al.* (1984) observed such a decrease in sunflower plants under water stress and in water-stressed *Ceratonia siliqua* reduction of leaf growth was also associated with cell wall stiffening (Rhizopoulou and Davies, 1991). In contrast, Van Volkenburgh and Boyer (1985) did not observe any significant change of cell wall extensibility in maize leaves as a result of water stress. However, Boyer *et al.* (1985) argued for the role of wall extensibility in the partial growth limitation of soybean stems.

Recent evidence suggests the involvement of a non-hydraulic signal, derived from roots growing in drying soil in the reduction of growth (Passioura, 1988; Saab and Sharp, 1989; Gowing *et al.*, 1990). This inhibition of growth seems to involve the

growth regulator, abscisic acid (ABA). Quarrie and Jones, (1977) demonstrated the inhibitory influence of ABA on leaf growth and it has also been shown to reduce cell wall extensibility (Van Volkenburgh and Davies, 1983; Kutschera and Schopfer, 1986) as a result of inhibition of proton secretion through the cell wall plasmalemma. The reduction of leaf growth in association with increased ABA in the xylem sap of maize plants subject to soil drying, but before any perturbation of shoot water status (Zhang and Davies, 1990a), has similarly been attributed to the role of ABA. Zhang and Davies (1990b) suggested that a negative log-linear relationship exists between leaf expansion and xylem sap ABA concentration in maize and sunflower plants subject to soil drying.

2.2.2 Stomatal conductance

Reduction of stomatal conductance in plants growing in conditions of limited soil water availability has been well established (see Hsiao, 1973). The traditional view of this response is that the reduction in water uptake by plants from dry soil induces lowering of leaf water potential which in turn causes a decline in turgor potential of stomatal guard cells. As a result, stomatal conductance is reduced. It has been shown that in many cases the reduction of stomatal conductance is associated with the decline of leaf water potential or turgor potential (Boyer, 1970; Jordan and Ritchie, 1971; Turner, 1974; Turner *et al.*, 1978; Ackerson *et al.*, 1980; Kelliher *et al.*, 1980; Henson *et al.*, 1983;). However, the influence of turgor potential on stomatal regulation is not always consistent (Schulze and Hall, 1982). For example, Jones and Rawson (1979) observed considerable differences in stomatal conductance at a constant turgor potential. This can be attributed to the influence of other microclimatic factors such as carbon dioxide concentration, photon flux density, temperature and water vapour saturation vapour pressure deficit, in association with leaf water status in the regulation of stomatal conductance (Jarvis, 1976). Turner *et al.* (1984) showed a direct influence of saturation deficit on stomatal conductance independent of changes in leaf water potential. Osonubi and Davies (1980) suggested that the stomatal sensitivity to saturation deficit is independent of soil water content although others have found that the response of stomatal conductance to saturation deficit is relatively less in plants growing in dry soil rather than in wet soil (Hall and Schulze, 1980, Johnson and Ferrell, 1983). Under limited soil water supply, a poor correlation has been reported between stomatal conductance and leaf water potential and turgor potential response to a changed in saturation deficit in both herbaceous and woody species whereas there was a close correlation between stomatal conductance and soil

water status (Turner *et al.*, 1985; Gollan *et al.*, 1985). Thus, stomatal conductance does not seem to be solely controlled by leaf water status, but may also be regulated by soil and/or root water status.

The closure of stomata during soil drying and before any detectable perturbation in leaf water status is now well documented for plants grown both in field and greenhouse environments (Bates and Hall, 1981; Blackman and Davies, 1985). Indeed, reduction in leaf conductance, as a result of soil drying, has been demonstrated even when shoot turgor was artificially maintained by pressurizing the roots (Gollan *et al.*, 1986). Masle and Passioura (1987) also found a reduction of transpiration when leaf water status remained unchanged, because of an increase in soil shear strength as soil dried. These results are consistent with the hypothesis that plant roots in drying soil generate a chemical signal that moves to the shoot through the transpiration stream. The decrease of cytokinin concentration or the increase of abscisic acid (ABA) concentration in roots of drying soil is thought to play an important role in such signalling.

Cytokinins are growth promoters that have long been implicated in enhancing cell division (Evans, 1984). The induction of stomatal opening in excised leaves by application of exogenous cytokinin (Meidner, 1967) demonstrated that an optimum concentration of cytokinin in leaves needs to be maintained for stomatal opening to occur. Livne and Vaadia (1972) showed reduction of cytokinin activity in shoots under water stress and Aspinall (1980) showed that the reduction of stomatal opening in water-stressed plants was associated with decreased cytokinin concentration in roots, leaves and xylem exudates. Cytokinin acts on the guard cell complex *via* membrane hyperpolarization (Incoll and Jewer, 1987). There is evidence that cytokinin is translocated from a site of bio-synthesis in the roots, to the shoot (Henson and Wareing, 1976). Blackman and Davies (1985) and Davies *et al.* (1986) showed that, when part of the root system of maize plants was water stressed, the stomatal conductance decreased although no changes in leaf ABA and leaf turgor were recorded. They suggested that this might result from reduction in cytokinin production in the roots and its translocation to the shoot. Incoll *et al.* (1990) observed reduction of cytokinin concentration in the apoplast close to stomatal guard cells when roots of *Phaseolus vulgaris* were subjected to soil drying.

In contrast, increased production of ABA in response to soil drying is widely accepted (Cornish and Zeevaart, 1985; Lachno and Baker, 1986; Zhang *et al.*, 1987). Unlike

cytokinin, abscisic acid (ABA) in plants is regarded as a growth inhibitor (Milborrow, 1966) that plays an important role in the control of stomatal opening in a fluctuating natural environment (Imber *et al*, 1974). The closure of stomata by endogenous ABA in water-stressed mesophytic plants has been shown by Loveys (1977). Abscisic acid is involved in stomatal regulation as shown by its action on epidermal strips (Snaith and Mansfield, 1982). Hartung (1983) and Hornberg and Weiler (1984) have suggested that the action of ABA is on the outer surface of the guard cell plasmalemma. Zhang and Davies (1989a) showed a close correlation between root ABA and surrounding soil moisture when maize plants were growing in a large, progressively-drying soil volume. The reduction of root tip turgor is thought to be the cause of production of ABA which eventually is transported to the shoot in the transpiration stream (Zhang and Davies, 1987; Neales *et al.*, 1989).

Zhang *et al.* (1987) demonstrated that increased concentration of leaf epidermal ABA and reduction of stomatal conductance of *Commelina* species occur when part of the root system is subjected to soil drying; they also indicated a good correlation with root ABA. Zhang and Davies (1987) observed an increased concentration of ABA in both leaf epidermis and mesophyll when root systems of *Commelina* were loaded with exogenous ABA. The reduction of stomatal conductance in response to increased leaf ABA has also been reported in the absence of any detectable change in leaf water status in plants subjected to soil drying (Grantz and Meinzer, 1990). Leaf synthesised ABA is generally trapped in the chloroplast and this ABA can also be released at the point of turgor loss (Pierce and Raschke, 1980). Leaf synthesized ABA could be transported to the roots in the phloem stream (Wolf *et al.*, 1990) and then into the transpiration stream, resulting in an additional increase of ABA in the xylem sap (Zhang and Davies, 1989b). However, ABA from leaf chloroplasts could also be released into the apoplast in the absence of turgor decline, because of a decreasing pH gradient between the cytoplasm and the apoplast when the pH of xylem sap is increased as a result of soil drying (Gollan *et al.*, 1992).

Since ABA is transported from roots in the transpiration stream, an increase in xylem sap ABA is likely to be the most immediate plant response to soil drying. Recent results suggest a significant role of xylem sap ABA on stomatal regulation (Zhang and Davies, 1989 a & b, 1990; Tardieu *et al.*, 1992b), since xylem sap has a direct link to the leaf apoplast, which is the leaf compartment closest to the stomatal guard cells. However, the role of xylem sap ABA in stomatal regulation in wheat plants has been questioned by Munns and King (1988). They failed to remove the antitranspirant

activity from xylem sap when ABA was removed (using an immunoaffinity column). The closure of stomata before any increase in xylem sap ABA occurred has also been reported in *Phaseolus vulgaris* when grown in a large soil volume drying gradually (Trejo and Davies, 1991). They suggested that some as yet unidentified substance in xylem sap was responsible for the control of stomatal action. However, Zhang and Davies (1991) did show the absence of antitranspirant activity in xylem sap of maize plants after ABA was removed (using an immunoaffinity column).

Since changes in ion fluxes across the guard cell membranes during stomatal regulation are well known, ions could have a direct influence on stomatal conductance. In particular, the role of the calcium ion in the regulation of stomatal conductance has been reported (Mcainish *et al.*, 1990). Analysis of xylem sap (Gollan *et al.*,1992) showed a decline in the concentration of nitrate, phosphate and calcium in association with a decline in soil moisture content. Reduction in the content of these ions during soil drying seems to modulate stomatal sensitivity to increased concentration of ABA in the xylem sap (Schurr *et al.*, 1992).

2.2.3 Photosynthesis, assimilate partitioning and root growth

Partial or complete inhibition of photosynthesis because of decline in capacity of the photosynthetic apparatus and stomatal closure as a result of water stress has long been known. In water-stressed plants, the initial reduction of photosynthesis is thought to be associated with the decline in leaf water potential (Boyer, 1971; Beardsell *et al.*, 1973; O' Toole *et al.*, 1977; Jones and Rawson, 1979; Ackerson and Herbert, 1981). The decline in leaf water potential reduces the photosynthetic capacity of chloroplasts to fix carbon dioxide (Mathews and Boyer, 1984) and increases resistance to carbon dioxide diffusion in the liquid phase from the mesophyll walls to the chloroplast (O' Toole *et al.*, 1976; Bunce, 1977). Sharp and Boyer (1986) showed that reduction in photosynthesis at low water potentials was a direct effect of water availability on chloroplast functioning.

Reports that soil drying induces stomatal closure before any change in leaf water status occurs (Bates and Hall, 1981; Blackman and Davies, 1985; Gollan *et al.*, 1986) can be explained in terms of stomatal limitation to photosynthesis under water stress. Stomatal closure protects plants against water loss but results in reduction of carbon dioxide assimilation. Several experiments have shown a strong correlation between stomatal conductance and net photosynthesis (Downton *et al.*, 1988; Srinivas Rao and

Bhatt, 1988; Metcalfe *et al*, 1989; Weber and Gates, 1990). The influence of increased concentrations of ABA on stomatal closure of water stressed plants is now well established (e.g. Zhang *et al.*, 1987) and so ABA may also be indirectly implicated in the regulation of photosynthesis in water-stressed plants. Thus it is not surprising that an ABA-induced decrease of RubisCo (ribulose 1,5-bisphosphate carboxylase-oxygenase) activity, linked to a decrease in net photosynthesis, has been reported (Sankhla and Hurber, 1974). Cornic and Miginiac (1983) also showed reduction of photosynthetic capacity of the mesophyll in association with reduced stomatal conductance when ABA was injected into the petiole of intact transpiring leaves.

Decreased development of leaf area can also reduce the total photosynthetic productivity of plants experiencing a water deficit. Because of immediate reduction of leaf expansion caused by water stress (Saab and Sharp, 1989; Gowing *et al.*, 1990), the photosynthetic area does not increase as in well-watered plants and this may lead to decrease in total photosynthetic productivity of water-stressed plants. Saab and Sharp (1989) observed no change in stomatal conductance and leaf water status of maize plants during soil drying, although leaf expansion declined, indicating a significant role of leaf area in determining photosynthetic productivity in their particular circumstances.

Partitioning of assimilates among plant parts is an important factor in plant growth and is thought to be changed by water stress (Jones *et al.*, 1981). When water supply is limiting allocation of assimilates tends to be modified in favour of root growth: root growth is enhanced and the root to shoot ratio increases (Hsiao and Acevedo, 1974). Significant increases in the root dry mass fraction has been reported in both water-stressed herbaceous and tree species (Osonubi and Davies, 1978; Sharp and Davies, 1979; Huck *et al.*, 1983; Steinberg *et al.*, 1990). This is an important adaptive mechanism when water is scarce that may enable plants to exploit maximum water availability in the soil profile. Sharp and Davies (1985) observed more soil water depletion from deep in the soil profile in association with higher root density there when maize plants were grown in long soil columns. An absolute increase of root dry matter in water-stressed plants in comparison with well-watered ones (Sharp and Davies, 1979) suggested a high capacity of the root for solute accumulation and turgor maintenance at low water potentials. Since shoot growth declines with partial stomatal closure while a substantial rate of carbon dioxide assimilation is maintained, the accumulating solutes may be available for allocation to the roots.

Karmoker *et al.* (1979) demonstrated translocation of sugars from shoot to root of intact plants of *Phaseolus vulgaris* loaded with ABA in the root system. A significant influence of ABA in enhancing root growth of maize, *Capsicum* and *Commelina* has also been reported (Watts *et al.*, 1981). Recent experimental evidences (e.g. Zhang and Davies, 1989a) showed that deep penetration of the root system during progressive soil drying was associated with increased concentration of ABA in roots and could be attributed to enhanced influence of ABA on root growth. In association with deeper penetration of the root system, the root length density also increased at lower levels under progressive soil drying (Klepper *et al.*, 1973; Sharp and Davies, 1985). Thus plants can explore deep soil layers in water scarce situations.

2.3 Root-shoot communication

Plants growing in nature interact with environmental perturbations through the functioning of their roots and shoots, which are also interrelated and interdependent in the control of growth and physiological activity. Any perturbation in the root environment can change root functioning which eventually may result in the transmission of a message to the shoot. There are two conflicting hypotheses in respect to this root-shoot communication in plants subjected to soil drying. When plants grow with a limiting soil water supply, it is commonly thought that there is a decline in shoot water status, thus inducing stomatal closure and reduced leaf growth rate (Kramer, 1983). This is the hydraulic signal. Alternately, the shoot could sense soil drying before any perturbation of shoot water status. Bates and Hall (1981) interpreted this non-hydraulic signal as a chemical signal from the roots.

According to the hydraulic hypothesis, as soil dries the roots experience a lower water potential that is rapidly transmitted to the shoot hydraulically through the xylem. It has been suggested (Westgate and Boyer, 1985) that, because of limited water in the root environment, tension is transmitted into the xylem resulting in a decline in the water potential gradient between xylem and growing cells and thus regulating growth. Boyer and Nonami (1990) demonstrated that a reduction in stem growth of soybean occurred as a result of such signaling when seedlings were transplanted from well-watered to poorly watered to water deficit vermiculite, although there was no evidence of an effect on stomatal regulation. The importance of hydraulic signaling can not be ruled out, at least in field grown plants (Kramer, 1988).

The observations of chemical signaling suggest that the root system senses soil drying by a reduction of root tip turgor leading to either a decrease or an increase in the level of a growth regulator which eventually is transmitted to the shoot in the transpiration stream. Such chemical signaling could be manifested as either negative or positive (Jackson and Kowalewska, 1983). The negative signal is thought to be the reduction in the cytokinin supply from roots growing in drying soil (Itai and Vaadia, 1965). In water-stressed plants, reduction of cytokinin activity induces leaf senescence, inhibition of protein synthesis and stomatal closure (Livne and Vaadia, 1972; Aspinall, 1980). Blackman and Davies (1985) suggested that the reduced cytokinin supply from roots in drying soil to the leaves results in the decline of stomatal conductance in maize plants, since stress-induced stomatal closure was alleviated by the application of cytokinin. In contrast, Neuman *et al.* (1990) were unable to demonstrate a reduction of cytokinin concentration in leaves of bean and hybrid poplar plants with hypoxic roots, although leaf growth and stomatal conductance both declined. However, another experiment (Incoll *et al.*, 1990), using *Phaseolus vulgaris* L. subjected to soil drying, showed a lower concentration of cytokinin in the xylem sap but no good correlation with stomatal closure.

More recently, it has been suggested that abscisic acid from dehydrating roots is transported to the shoot in the transpiration stream to control shoot activity (Zhang and Davies, 1987; Neales *et al.*, 1989); this is the positive signal. Closure of stomata and reduction of leaf growth rate have been demonstrated to occur as a result of such signaling from water-stressed roots of many species growing in the greenhouse, e.g. *Commelina*, sunflower and maize (Zhang *et al.*, 1987; Zhang and Davies, 1989a, 1989b, 1990a, 1990b) and also in the field, e.g. almond and maize (Wartinger *et al.*, 1990; Tardieu *et al.*, 1992b; Schurr *et al.*, 1992). However, bean plants subjected to soil drying in a greenhouse experiment (Trejo and Davies, 1991) did not show a substantial increase in xylem sap abscisic acid, despite stomatal closure. This could be attributed to the involvement of other compounds, resulting in a positive signal. Munns and King (1988) demonstrated that there is another unknown potential inhibitor of transpiration in the xylem sap of wheat plants.

2.4 Soil water deficit and agroforestry

Efficient use of environmental resources and sustainability are more important criteria of land-use systems such as agroforestry than absolute yield (see Vose, 1981). In a discussion of plant response to soil drying it is important to pay attention to its implications for mixed stands, such as in agroforestry systems (Connor, 1983). The key to effective agroforestry systems, in areas where soil water is limiting, is the efficient use of water throughout the soil profile. Changes in root and shoot behaviour of component species may play a vital role in such water extraction, leading to the maintenance of sustainability in an agroforestry system. For example, an increase in the root/shoot ratio (Sharp and Davies, 1979) and deep penetration of the root system (Sharp and Davies, 1985), together with differential characters of the component species could ensure maximum exploration throughout different levels of the soil profile. Alternatively, earlier reduction of stomatal conductance and leaf expansion (Bates and Hall, 1981; Blackman and Davies, 1985; Gollan *et al.*, 1986; Passioura, 1988) could decrease transpiration loss. The combination of these two mechanisms may well result in sustained utilization of available soil water in an area of water shortage and thus the maintenance of productivity. However, reduction in productivity as a result of competition for water between component species has also to be considered (Berendse, 1979; Thomas, 1984, Jonsson *et al.*, 1988; Singh *et al.*, 1989).

Competition between annual crops may often be minimal because of temporal and spatial separation. However, in an area of limited surface soil water the horizontal expansion of tree roots (Kummerow, 1980) and deep penetration of crop roots (Sharp and Davies, 1985) could potentially lead to competition for water between the component species. In contrast, Mathavan *et al.* (1985) showed improvement of soil moisture availability in the shallow soil layer in a tea/clover mixture, during occasional drought. This could be attributed to transport of water from the deeper, moist sub-soil to the shallower soil surface layer by the deep-rooted component, thus making water available for the shallow-rooted component (Corak *et al.*, 1987). In other cases, the reduction of total root development during soil drying (Sharp and Davies, 1985) may also minimize competition, because of possible decline in root density. Eastham and Ross (1988) reported reduction of competition in a pasture (*Setaria sphacelata* cv. kazungula)/ young tree (*Eucalyptus grandis*) combination because of lower root density. However, reduced development of the root system might also lead to reduction in the competitive ability of the component species (Thomas, 1984).

In an agroforestry system, competition between the component species for below-ground water can affect the relationship between soil and leaf water status. For example, lower leaf water potentials and leaf conductances were found in *Encelia farinosa* when the plants were grown with neighbours than without neighbours, indicating limitation by soil water because of competition between the component species (Ehleringer, 1984). However, during soil drying, early stomatal closure (Blackman and Davies, 1985) and reduction in leaf expansion (Passioura, 1988), before any decline in leaf water potential can reduce water loss, may result in the conservative use of available soil water. As a result, in the case of annuals, soil water is not usually exhausted before the completion of reproductive growth (Gollan *et al.*, 1986; Masle and Passioura, 1987). Similar mechanisms may be adopted by trees to overcome drought periods with sustained productivity. Such a mechanism may also enable the optimization of growth according to the amount of water in the soil (Jones, 1980; Cowan, 1982). It is possible, therefore, that competition between component species for water in an agroforestry system in a water shortage area could be potentially minimal, resulting in the maintenance of the sustained productivity that is an important aspect of agroforestry. This possibility needs to be investigated.

CHAPTER 3

Growth and Competitive ability of Bean and Poplar in a Simulated Agroforestry System in Relation to Soil Water Supply

3.1 Introduction

Since the availability of soil water is uncertain, particularly in monsoon and savanna climates (Monteith,1977), rainfed terrestrial plants are rarely free from water stress (Hsiao *et al.*, 1976). Knowledge of responses of annuals and young trees to soil water is important in choosing a good combination in an agroforestry system, so as to avoid the negative consequence of the system. Poplar is well accepted as an agroforestry species in a number of countries, because of its intensive culture in short-rotation biomass production as well as its excellent growth on a wide range of sites (Ceulemans, 1990). French dwarf bean is a successful crop for food and forage in areas of medium rainfall from the tropics to temperate regions. It is usually grown as an intercrop with maize, sweet potato, cotton, coffee etc. in tropical Africa (see Purseglove, 1968). Both bean and poplar are sensitive to water stress (e.g. Kelliher *et al.*, 1980; Schulte *et al.*, 1987; Hegde and Srinivas, 1990) and in that respect are typical of a wide range of agricultural and tree crops. In some intercropping experiments French dwarf bean has shown complementary effects (e.g. Chingaipe, 1985; Maghembe *et al.*, 1986) but in other cases the legume yield has been reduced by competition (Francis *et al.*, 1986). The responses of mixed stands of bean and poplar need to be investigated under different soil water regimes to identify the circumstances in which they show compatible and complementary characteristics. Studies on shoot and root growth, as well as on shoot water relations, are also desirable in this respect.

The present study was conducted to investigate the growth, yield and water relations parameters of an agricultural crop plant and a young tree, growing in monoculture and in mixed stands under three different soil water supply regimes in the greenhouse: yield ability and competitive performance of both species have also been taken into consideration. Although the greenhouse differs from the field (e.g. small plot size, reduced insolation, limited rooting depth and high temperatures), responses to water shortage may be similar (Thomas and Norris, 1981).

3.2 Materials and Methods

3.2.1 Plant materials and experimental design

Pre-rooted cuttings (about 8 weeks of age) of poplar (*Populus trichocarpa* X *p.deltoides* cv. Raspalje) were transplanted into black tubes (15 cm diameter and 75 cm depth), prepared from drain pipe, which were filled to 72 cm with compost of a mixture of 50% loam soil, 25% sand and 25% peat. The pH of the compost was recorded as 6.6 ± 0.06 (n = 8). Seeds of French bean (*Phaseolus vulgaris* L. cv. Argus) were sown directly into the tubes. All tubes were watered every alternate day until the cuttings of poplar and the seedlings of bean were established. Poplar cuttings with new leaves and bean seedlings with nearly fully expanded first trifoliate leaf were considered as established plant stocks. In monoculture the number of plants in each pot was kept to one, either poplar or bean, whereas in mixed stands there was one poplar and one bean in each pot. The design of the experiment was a completely randomized block with five blocks and nine treatments as follows: three cropping systems (bean grown in monoculture; poplar grown in monoculture; bean and poplar grown in mixed stands) each with three water supply regimes (watered every alternate day, i.e. watered control = WC; watered at weekly intervals; i.e. rewatered = RW; unwatered from day 1 = UW). The treatments were started from the date of establishment of the plant stock. The experiment was continued for three consecutive drying cycles of treatment UW with six days of regular watering (every alternate day) inbetween each cycle. The first drying cycle was for 12 days and the two subsequent cycles of 14 days each. The first drying cycle took place before flowering of the bean plants.

3.2.2 Light, temperature and vapour pressure deficit

Daily photosynthetic photon flux density (DPPFD) incident on the experimental area was recorded using a quantum sensor (calibrated against a standard quantum sensor, LI-190 SB, Li-Cor Inc., Lincoln, USA) and a data logger (Deltalogger, Delta-T Devices Co. Ltd., Cambridge, UK). Using thermocouples (copper-constantan thermocouple wire, British standard B10 BS 1843 Type T, T. C. Ltd., Uxbridge, UK) 'dry-bulb' temperatures T_a and 'wet-bulb' temperatures T_w were also measured. The vapour pressure deficit (∂e) was calculated as:

$$\partial e = e_s(T_a) - e$$

where $e_S(T_a)$ = saturation vapour pressure (kPa) at T_a, and e = actual vapour pressure (kPa).

$$e = e_S(T_w) - \gamma(T_a - T_w)$$

where $e_S(T_w)$ = saturation vapour pressure (kPa) at T_w, and γ = the psychrometric 'constant', taken as 0.08 kPa $°C^{-1}$ for the unaspirated 'wet-bulb'.

Hourly averages were obtained as an average of readings recorded at 10 minute intervals. These data were recorded for a period of 30 days from the begining of the second drying cycle to the 10th day of the third drying cycle.

3.2.3 Data collection and analyses

The water content of four different layers (0-18; 18-36; 36-54; 54-72 cm) of the soil columns was monitored on days 1, 6 and 11 during the first and second drying cycles. The soil samples were collected in two replicates, using a 13 mm diameter metallic corer. Fresh and oven dry (at 105 °C for 24 hours) weights of the samples were recorded and the water content of the soil calculated.

Stem length of the plants in all treatments was measured on days 1, 6 and 12 of the first cycle and on days 1, 6 and 13 of the second drying cycle. In the case of French bean, during the first drying cycle the entire shoot length from the soil surface was measured, and during the second drying cycle the length of a typical growing shoot developed from a node was measured. In the case of poplar the shoot developed from the cutting was measured during both drying cycles. Stem length increment was calculated by subtracting the initial length from the length of the following measurement.

Length and width of the last fully expanded leaf were measured on the same day as stem length during the first and the second drying cycles in all treatments. In bean, only the terminal leaflet of a trifoliate leaf was measured (Ndawula-Scnyimba, 1972). These measurements were converted into areas, using regression equations ($Y = -5.2776 + 1.5353X$, $r^2 = 0.998$, for bean and $Y = -4.7671 + 0.6314X$, $r^2 = 0.998$, for poplar), developed by plotting actual leaf area (measured by a leaf area meter, LI 3100, Li Cor Inc., Lincoln, USA) against the product of length and width of a number (18 and 19, respectively) of leaves.

Leaf water potential of the last fully expanded leaves (three from each treatment) was measured using a pressure chamber (Scholander *et al.*, 1965), on day 12 of the first cycle and day 11 of the second drying cycle at mid-day and pre-dawn, respectively. During the third drying cycle, leaf water potential was also measured at mid-day on days 2, 7 and 14. The 5th or 6th leaf of poplar from the stem apex and the terminal leaflet of bean (at approximately the same height from the soil surface) were taken for measurement.

Abaxial stomatal conductance of the last fully expanded leaves (four from each treatment) was measured after five to six hours of photoperiod, using an automatic diffusion porometer (Delta-T Devices Co. Ltd., MK II, Cambridge, UK.) on day 12 of the first and day 11 of the second drying cycle and on days 1, 6 and 12 during the third drying cycle. A perforated, polypropylene calibration plate was used as a standard to calibrate the porometer each day it was used. The calibration was in the form of a regression equation. The conductance was corrected for temperature using the method described in the manual.

At the end of the third drying cycle, because of abnormal appearance (i.e. yellowing and drying of stem and leaf) of a few plants in one block, the other four replicates of each treatment were harvested and the roots, stems, leaves and, in the case of bean, the fruits separated. The total leaf area of each plant was measured, using a leaf area meter (LI-3100, Li Cor Inc., Lincoln, USA). During harvesting of the root system, the entire soil column of two replicates of each treatment was divided into four sections of 18 cm. The roots in each section were collected separately, with the poplar and bean roots also being separated in the mixed culture treatment. In the remaining two replicates of each treatment the entire root system of bean and poplar was separated from the soil, whether grown in monoculture or in mixed culture, before being divided into four sections of 18 cm length. These two methods of harvesting roots were used to identify the root systems of the two species and to compare estimates of the amount of roots of each species (particularly in mixed culture) in each section by these two methods, so as to harvest the roots with minimum error. The fine roots of each species (excluding coarse roots of *ca* 3 mm diameter or above for bean and 4 mm or above for poplar) in each section were also separated. Roots, stem and leaves as well as the fruits of bean, were oven dried at 80 °C for seven days and their dry weights recorded. From these data, leaf area ratio (LAR, leaf area/total plant dry weight), leaf weight ratio (LWR, leaf dry weight/total plant dry weight), stem weight ratio (SWR, stem dry weight/total plant dry weight), root

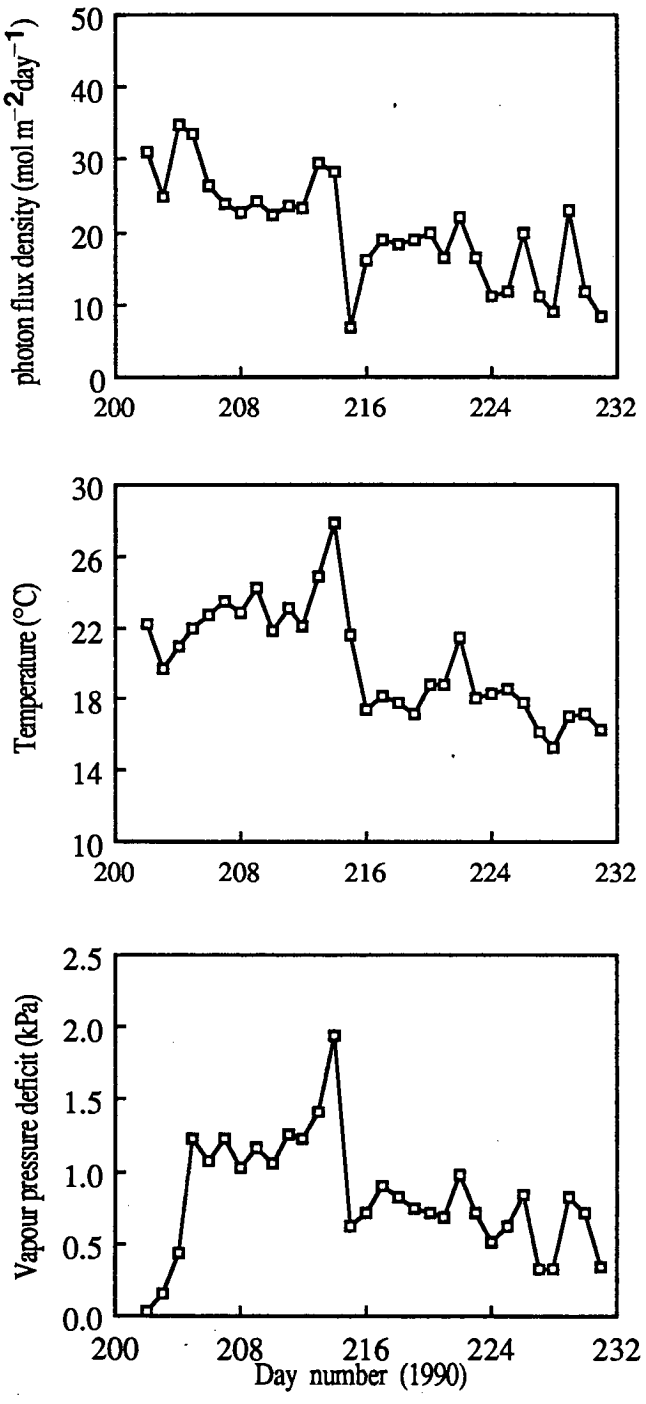

Figure 3.1: Mean photosynthetic photon flux density, temperature, and vapour pressure deficit on the experimental plot over a period of 30 days, from the beginning of the second drying cycle to the 10th day of the third drying cycle for the 14 hour photoperiod.

weight ratio (RWR, root dry weight/total plant dry weight) and fruit weight ratio (FWR, fruit dry weight/total plant dry weight) of bean were derived. The specific leaf area (SLA, leaf area/leaf dry weight) was also calculated.

Relative yield (RY) (yield of mixed culture relative to monoculture) of each species was calculated as x/y, where x and y are the yields in mixed stands and monoculture, respectively. From this, the relative yield total (RYT) of the mixture was derived as the sum of x/y for the two component species (Thomas, 1984). Competitive performance (C) of both species was calculated as $C = \ln(2x/y)$, where positive values of C indicate competitive enhancement and negative values indicate competitive suppression (Rhodes, 1981; Thomas,1984).

Nitrogen, phosphorus and potassium contents of leaf samples from watered control plants of each species, grown in both monoculture and mixed culture, were assessed to test whether any reduction of dry matter production in mixed stands could be ascribed to nutrient limitation.

The growth and yield data were analysed by analysis of variance using MINITAB, followed by the calculation of LSD. The results of the NPK analyses were tested by simple paired t-tests using the STATVIEW package.

3.3 Results

3.3.1 Light, temperature and vapour pressure deficit

During the 30 days of measurement the range of daily mean photosynthetic photon flux density during daylight hours was 7-35 mol m^{-2} day^{-1}. The range of mean temperature was 15-28 °C with mean vapour pressure deficit 0.33-1.94 kPa (Fig. 3.1).

3.3.2 Soil water content

In the first drying cycle, significant reduction in soil water was restricted to the top 36 cm when bean and poplar were grown in monoculture, but in the mixed stands the reduction extended below 36 cm depth of soil column in treatment UW (Fig. 3.2 left). In the second drying cycle, when bean was grown in monoculture the reduction in soil

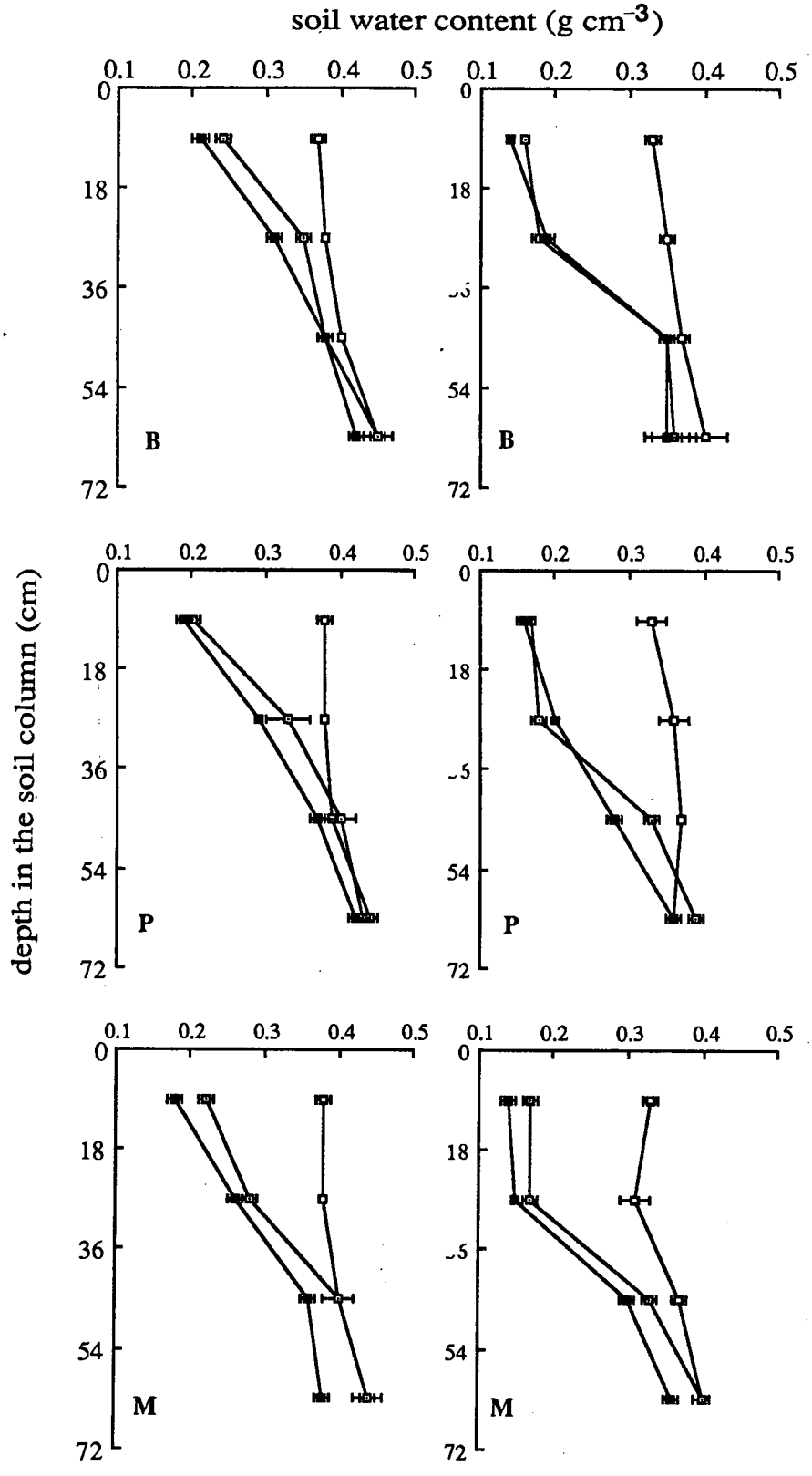

Figure 3.2: Water content at various depths in the soil column, for bean and poplar, grown in monoculture (B & P, respectively) and in mixed stands (M) on the 11th day of the first (left) and the second (right) drying cycles under three.different soil water supply regimes. (□) watered control WC; (⊡) re-watered RW and (■) unwatered UW. Points are means of two measurements ± one standard error of the mean.

water content was also restricted to the top 36 cm, whereas in the monoculture of poplar and in the mixed stands, reduction could be seen at 54 cm and 72 cm, respectively (Fig. 3.2 right). Irrespective of the cropping system, there was a larger reduction in soil water content in the upper 36 cm than throughout the whole soil column (0-72) in both drying cycles (Fig. 3.2). The water supply treatments were successful in imposing different degrees of water stress on the plants, although differences between treatments RW and UW were small in the second drying cycle. Comparing the cropping systems, there were larger reductions of soil water in the mixed stands of bean and poplar than in the monocultures.

3.3.3 Total biomass

Total biomass production of bean plants when grown in mixed stands was unaffected by limited water supply, but in monoculture the total biomass production was significantly reduced in treatment UW relative to treatment WC (Table 3.1A). In poplar, total biomass production was significantly reduced by limited water supply when grown either in monoculture or in mixed stands (Table 3.2A). However, the reduction in poplar total biomass production resulting from treatments RW and UW was not significant in the mixed stands. Although total biomass production of each species was significantly reduced in the mixed stands relative to the monoculture, in treatment UW the difference was not significant in the case of the bean. These results indicate good performance of both bean and poplar plants in mixed stands under severely limited soil water supply.

3.3.4 Root biomass

There was no significant effect of soil water supply on root biomass production when bean was grown either in monoculture or in mixed stands with poplar. However, in the watered control treatment WC, bean root biomass was significantly more reduced in the mixed stands than in the monoculture (Table 3.1A). When expressed as a ratio with total biomass (RWR), bean RWR was even larger in treatment UW than in treatments RW and WC (Table 3.1B). By contrast in poplar root biomass production was significantly reduced by limited soil water supply when grown either in monoculture or in mixed stands, so that poplar RWR was smaller in treatments UW and RW than in the watered control WC (Table 3.2). In the limited water supply treatments UW and RW, root biomass production of beans in mixed stands was

Table 3.1: Effect of soil water regime on yield of bean (*Phaseolus vulgaris* L.) grown in monoculture (mo) and mixed stands with poplar (mi) after three consecutive drying cycles. Analysis by two way block ANOVA (see Appendix I). Mean of four observations ± one standard error of mean. Watered control (WC); re-watered (RW); unwatered (UW); treatment (T); cropping system (C) and interaction between T & C (I); root weight ratio (RWR); stem weight ratio (SWR); leaf weight ratio (LWR); pod weight ratio (PWR); leaf area ratio (LAR); specific leaf area (SLA).

(A) Total

Variable	Water supply regime							Significance		
	WC		RW		UW		LSD	T	C	I
	mo	mi	mo	mi	mo	mi	(p<0.05)	(p<)	(p<)	(p<)
Root dry wt (g)	4.18 ±0.39	2.69 ±0.18	3.48 ±0.28	2.93 ±0.34	3.81 ±0.29	3.07 ±0.37	0.94	ns	0.01	ns
Stem dry wt (g)	18.01 ±1.43	13.09 ±1.93	13.18 ±1.61	9.99 ±1.33	9.08 ±0.53	7.72 ±0.49	4.54	0.01	0.01	ns
Leaf dry wt (g)	13.72 ±1.34	8.51 ±0.86	12.91 ±1.92	10.07 ±0.97	10.47 ±0.92	9.38 ±0.34	3.40	ns	0.01	ns
Fruit dry wt (g)	59.77 ±5.01	35.41 ±4.24	46.97 ±5.58	36.86 ±3.51	38.08 ±3.22	31.27 ±2.35	11.99	0.05	0.001	ns
Total dry wt (g)	95.68 ±8.03	59.55 ±6.73	79.24 ±8.80	59.86 ±5.27	61.46 ±3.64	51.44 ±3.37	17.89	0.01	0.001	ns
Leaf area (dm^2)	49.90 ±4.13	34.20 ±3.93	43.45 ±4.37	36.25 ±3.75	31.98 ±1.55	33.40 ±2.19	10.35	0.05	0.05	ns

(B) Proportion

RWR ($g\ g^{-1}$)	0.04 ±0.01	0.04 ±0.01	0.04 ±0.01	0.05 ±0.01	0.06 ±0.01	0.06 ±0.01	0.02	ns	ns	ns
SWR ($g\ g^{-1}$)	0.19 ±0.004	0.22 ±0.01	0.17 ±0.01	0.17 ±0.01	0.15 ±0.01	0.15 ±0.01	0.03	0.001	ns	ns
LWR ($g\ g^{-1}$)	0.14 ±0.01	0.14 ±0.004	0.16 ±0.01	0.17 ±0.01	0.17 ±0.01	0.18 ±0.01	0.03	0.01	ns	ns
PWR ($g\ g^{-1}$)	0.62 ±0.01	0.59 ±0.02	0.62 ±0.01	0.61 ±0.01	0.62 ±0.02	0.61 ±0.01	0.04	ns	ns	ns
LAR ($dm^2\ g^{-1}$)	0.52 ±0.01	0.57 ±0.01	0.55 ±0.01	0.60 ±0.02	0.52 ±0.02	0.65 ±0.01	0.04	0.05	0.001	0.05
SLA ($dm^2\ g^{-1}$)	3.65 ±0.12	4.00 ±0.13	3.45 ±0.20	3.60 ±0.12	3.09 ±0.18	3.55 ±0.12	0.39	0.01	0.01	ns

Table 3.2: Effect of soil water regime on yield of poplar (*Populus trichocorpa X P. deltoides* cv. Raspalje) grown in monoculture (mo) and mixed stands with bean (mi) after three consecutive drying cycles. Analysis by two way block ANOVA (see Appendix I). Mean of four observations ± one standard error of mean. Watered control (WC); re-watered (RW); unwatered (UW); treatment (T); cropping system (C) and interaction between T & C (I); root weight ratio (RWR); stem weight ratio (SWR); leaf weight ratio (LWR); pod weight ratio (PWR); leaf area ratio (LAR); specific leaf area (SLA).

(A) Total

Variable	Water supply regime							Significance		
	WC		RW		UW		LSD	T	C	I
	mo	mi	mo	mi	mo	mi	(p<0.05)	(p<)	(p<)	(p<)
Root dry wt (g)	24.34 ±2.23	15.20 ±1.32	12.90 ±0.75	4.81 ±0.50	9.11 ±0.54	4.18 ±0.15	3.56	0.001	0.001	ns
Stem dry wt (g)	30.05 ±2.62	15.98 ±2.03	20.83 ±2.21	8.73 ±1.23	13.47 ±0.79	6.74 ±1.35	5.60	0.001	0.001	ns
Leaf dry wt (g)	41.44 ±3.72	21.90 ±1.72	25.87 ±2.22	11.19 ±1.80	17.39 ±0.76	8.66 ±1.89	6.89	0.001	0.001	ns
Total dry wt (g)	95.83 ±8.08	53.07 ±3.46	59.59 ±5.11	24.73 ±3.35	39.97 ±1.88	19.58 ±3.27	14.80	0.001	0.001	ns
Leaf area (dm^2)	67.53 ±3.32	37.13 ±4.24	46.25 ±4.07	22.25 ±2.91	29.00 ±1.25	17.73 ±3.38	9.95	0.001	0.001	0.05
(B) Proportion										
RWR ($g\ g^{-1}$)	0.25 ±0.01	0.29 ±0.03	0.22 ±0.01	0.20 ±0.02	0.22 ±0.01	0.23 ±0.03	0.06	0.05	ns	ns
SWR ($g\ g^{-1}$)	0.32 ±0.01	0.30 ±0.02	0.35 ±0.01	0.35 ±0.02	0.34 ±0.01	0.34 ±0.01	0.04	0.05	ns	ns
LWR ($g\ g^{-1}$)	0.43 ±0.01	0.41 ±0.02	0.43 ±0.02	0.45 ±0.01	0.44 ±0.01	0.43 ±0.02	0.04	ns	ns	ns
LAR ($dm^2\ g^{-1}$)	0.71 ±0.03	0.70 ±0.05	0.78 ±0.02	0.90 ±0.02	0.73 ±0.01	0.84 ±0.03	0.08	0.01	0.01	0.05
SLA ($dm^2\ g^{-1}$)	1.65 ±0.08	1.68 ±0.08	1.79 ±0.05	2.02 ±0.09	1.67 ±0.04	1.96 ±0.07	0.20	0.01	0.01	ns

almost the same as in monoculture while poplar root biomass production was significantly lower in mixed stands than in monoculture.

3.3.4.1 Feeding root distribution at different depths in the soil column

The root system of bean plants in treatment UW extended below 60 cm, compared with a maximum of 50 cm in treatments RW and WC. A similar rooting pattern was observed whether beans were grown in monoculture or in mixed stands, although in monoculture, the root system was slightly more extensive than in mixed stands at all depths in the soil column (Fig. 3.3 left). Similarly, *ca* 45% of bean root weight in treatment UW occurred in the top 18 cm of the soil column compared with *ca* 60% in the watered control WC (data not shown).

When poplar plants were watered frequently, *ca* 90% of the root weight occurred in the top 50 cm of the soil column, whereas in treatments RW and UW root weight was almost uniformly distributed to a depth below 65 cm (Fig. 3.3 right). As with beans, poplars grown in monoculture showed a similar rooting pattern to poplars grown in mixed stands. However, there were significantly more roots at all depths in the monoculture.

3.3.5 Stem growth

As expected, growth of stem length of both bean and poplar was reduced in treatments RW and UW. Differences between RW and UW were generally small and inconsistent although the difference was highly significant ($P < 0.0001$) in mixed stands in the second drying cycle (Fig. 3.4). Differences in stem growth between monoculture and mixed stands in the well-watered control were small and insignificant for both species. However, stem growth of beans in the mixed stands compared to the monoculture was *ca* 82% and *ca* 75% for treatments RW and UW, respectively, averaged over two drying cycles, and for poplar *ca* 81% and *ca* 52% (data not shown), respectively.

3.3.6 Stem biomass

Stem biomass production of bean when grown alone was significantly reduced in the limited water supply treatments RW and UW but in mixed stands stem biomass reduction was observed only in treatment UW (Table 3.1A). When expressed as a

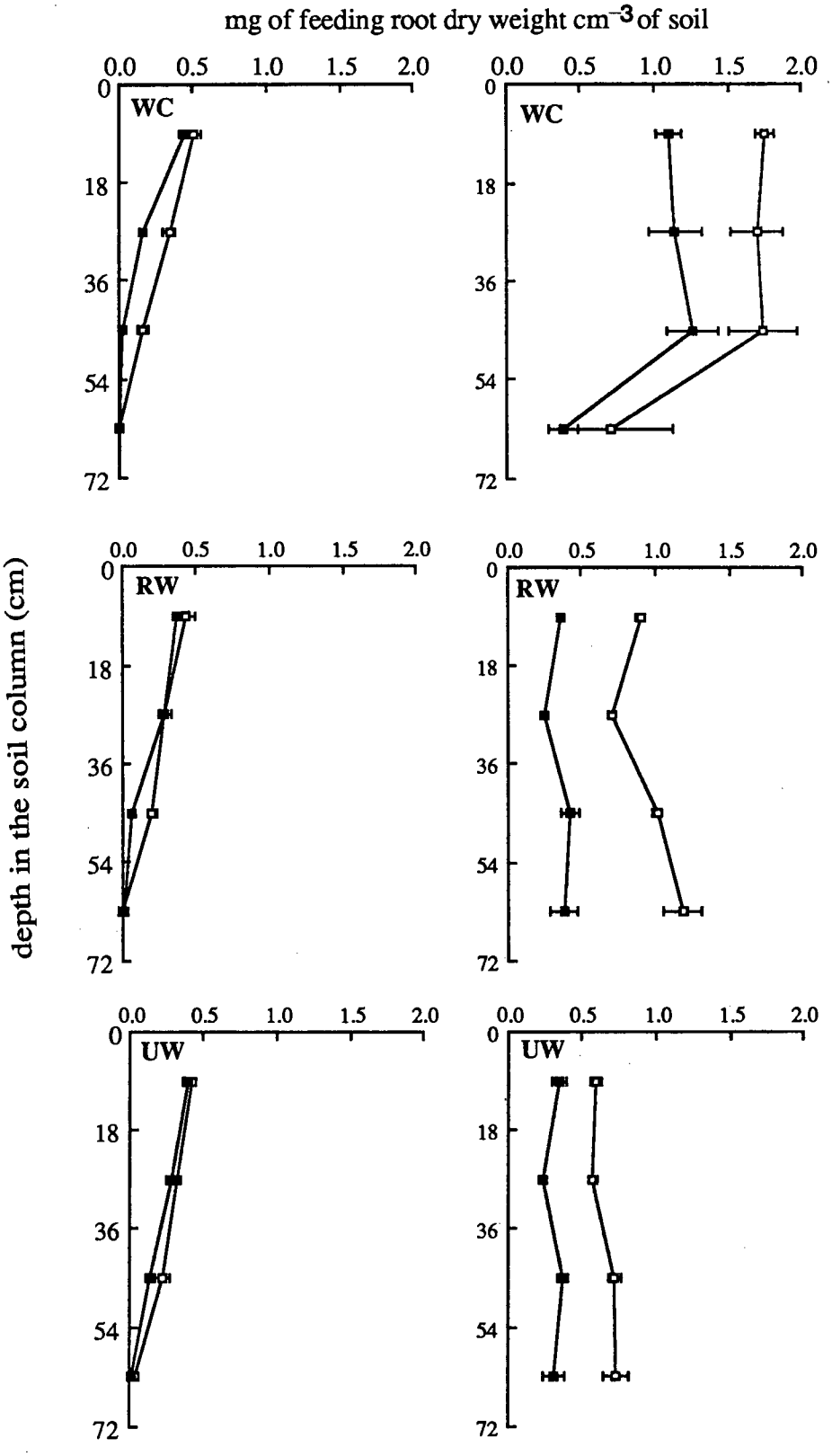

Figure 3.3: Dry weight distribution of bean (left) and poplar (right) feeding roots, grown in monoculture (□) and in mixed stands (■), down the soil column at the end of three consecutive drying cycles under three different soil water supply regimes. (WC) watered control; (RW) re-watered and (UW) unwatered. Points are means of four observations ± one standard error of mean.

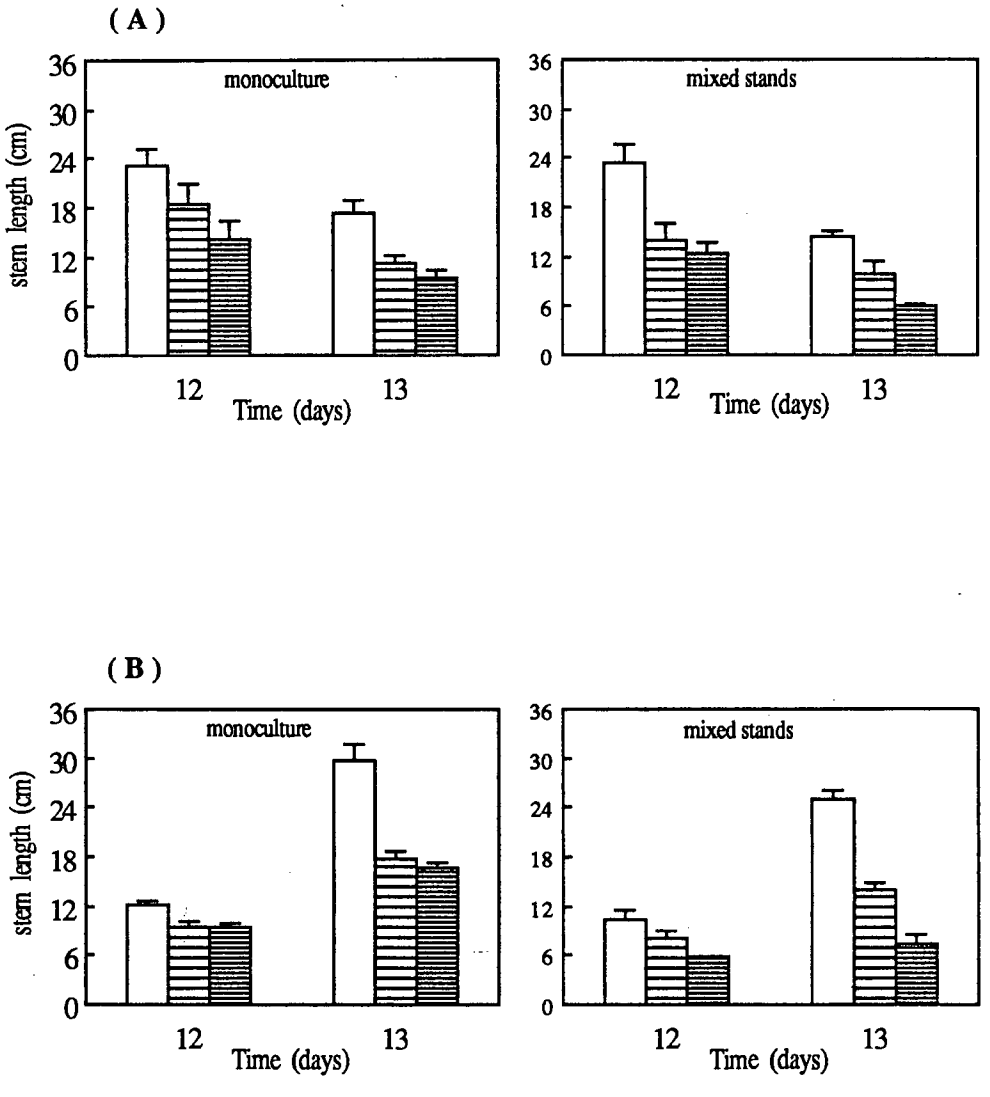

Figure 3.4: Increase in stem length of bean (A) and poplar (B), grown in monoculture (left) and in mixed stands (right), over a period of 12 and 13 days in the first and the second drying cycles, respectively under three different soil water supply regimes. (□) watered control WC; (▤) re-watered RW; (▤) unwatered UW. Columns are means of five observations ± one standard error of mean.

ratio with total biomass (SWR), SWR was also significantly less in treatment UW when grown in monoculture or in mixed stands and in treatment RW in the mixed stands (Table 3.1B). Stem biomass production of bean plants was less in the mixed stands than in the monoculture at all levels of water supply, but the difference was only significant in the well-watered control.

The reduction of stem biomass production of poplar caused by limited water supply was highly significant when grown either in monoculture or in mixed stands but only in the monoculture was this reduction significantly larger in treatment UW than in treatment RW (Table 3.2A). However, poplar SWR was even larger in treatments RW and UW than in the watered control WC (Table 3.2B). Stem biomass production of poplar was significantly reduced in mixed stands at all levels of soil water compared to the monoculture, although SWR was unaffected (Table 3.2).

3.3.7 Leaf expansion

The size of the last fully expanded bean leaf was significantly ($P < 0.0001$) reduced (almost 50%) when soil water supply was severely limited in treatment UW compared with the watered control WC and the treatment RW at the end of the first drying cycle. This effect was similar whether bean was grown in monoculture or in mixed stands (Fig. 3.5A). In mixed stands the last fully expanded leaf was slightly larger under limited water supply than in monoculture. During the second drying cycle, however, leaf size was not significantly reduced under water stress in monoculture, and indeed in mixed stands leaf size in treatments RW and UW appeared to be similar to that of the watered control plants. However, by the end of this cycle leaf size was reduced in all water supply treatments (Fig. 3.5A).

The last fully expanded leaf of poplar was significantly smaller (almost 20%) in treatment UW towards the end of the first ($p < 0.05$) and the second ($p < 0.0001$) drying cycles in monoculture as well as in mixed stands (Fig. 3.5B). Only in mixed stands was the effect of treatments RW and UW on leaf expansion significantly different at the end of the second drying cycle (Fig. 3.5B right). Irrespective of soil water supply there were no significant differences in leaf expansion between monoculture and mixed stands.

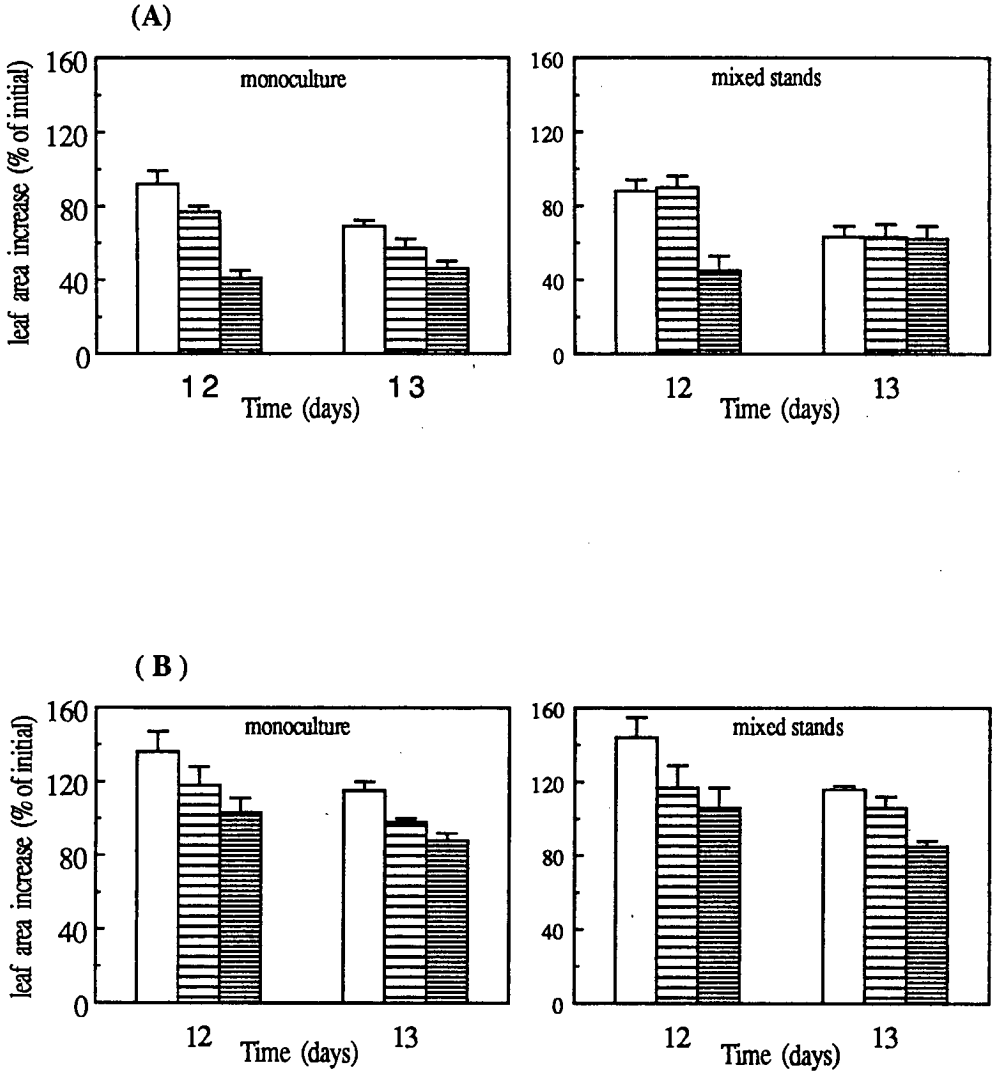

Figure 3.5: Leaf area increase of bean (A) and poplar (B), grown in monoculture (left) and in mixed stands (right), over a period of 12 and 13 days in the first and the second drying cycles, respectively, under three different soil water supply regimes. (□) watered control WC; (▤) re-watered RW; (▤) unwatered UW. Columns are means of five observations ± one standard error of mean.

3.3.8 Total leaf area

Total leaf area of bean was significantly less in treatment UW than in treatments RW and WC only when grown in monoculture (Table 3.1A), possibly as a consequence of reduced final leaf size (Fig. 3.5A). Irrespective of soil water supply, bean leaf area ratio (LAR) was significantly larger in mixed stands than in monoculture and comparatively much larger in treatment UW than in the treatments RW and WC (Table 3.1B). The significant interaction term in the ANOVA indicates the combined influence of water supply and cropping system.

In poplar, the reduction of total leaf area was highly significant in treatments RW and UW, whether grown in monoculture or in mixed stands, and there was no significant difference between treatments RW and UW when grown in the mixed stands (Table 3.2A). However, in all cases total leaf area was significantly less in mixed stands than in monoculture. Compared to the monoculture, poplar LAR was significantly larger in the mixed stands under water stress (Table 3.2B). For both total leaf area and LAR, the significant interaction term indicates the combined influence of water supply and cropping system (Table 3.2).

3.3.9 Leaf biomass

Leaf dry weight accumulation of bean was unaffected by water stress whether grown in monoculture or in mixed stands and it was significantly smaller in mixed stands than in monoculture only in the watered control treatment WC (Table 3.1A). In both the limited water supply treatments RW and UW, leaf weight ratio (LWR) was larger than in the watered control (Table 3.1B).

Like total leaf area, leaf dry weight accumulation of poplar was also significantly smaller under water stress and, compared to the monoculture, leaf dry weight was significantly smaller in mixed stands at all levels of soil water supply (Table 3.2A). In the mixed stands, there were no significant effects of the treatments RW and UW in this respect. Poplar LWR was unaffected by water stress (Table 3.2B).

3.3.10 Specific leaf area

Specific leaf area (SLA) of bean plants was significantly smaller in treatment UW both in monoculture and mixed stands and in treatment RW in mixed stands than in treatment WC (Table 3.1B).

SLA of poplar increased with water stress in mixed stands and was larger in mixed stands than in monoculture (Table 3.2B).

3.3.11 Pod biomass of bean

Pod biomass production was significantly less in treatment UW in monoculture, whereas in the mixed stands it was unaffected by water stress although significantly less in mixed stands than in monoculture, except in the treatment UW (Table 3.1A). However, pod weight ratio (PWR) was unchanged in any treatment (Table 3.1B).

3.3.12 Relative yield and relative yield total

The yield of bean and poplar plant parts (root, stem and leaf, as well as pods of bean) in the mixed stands relative to the monocultures (RY) was unaffected by limited water supply. Poplar yield was reduced more than bean yield in relative terms, when grown in mixed stands (Table 3.3A). The relative yield of bean in treatment WC was less than in treatments RW and UW and comparable to that of poplar, for which the relative yield was less in treatments RW and UW than in treatment WC. However, the relative total leaf area of poplar was similar at all levels of soil water supply (Table 3.3A).

Relative yield total (RYT) was not significantly affected by water stress and for all plant parts (root, stem and leaf) RYT was significantly more than 1.00 at all levels of soil water supply (Table 3.3B), largely because of the high relative yield of the bean plants. In both treatments RW and UW, the RY contribution of bean plants to RYT was significant (Table 3.3A).

3.3.13 Competitive performance

The competitive performance of bean was clearly greater than that of poplar when water supply was limited for all four measures of performance (total leaf area, root, stem and leaf biomass) (Table 3.4). In all cases except root weight, the competitive

Table 3.3: Effect of soil water regime on relative yield and relative yield total of bean and poplar grown in mixed stands after three consecutive drying cycles. Analysis by two way block ANOVA (see Appendix I). Mean of four observations ± one standard error of mean. Watered control (WC); re-watered (RW); unwatered (UW); treatment (T); species (S) and interaction between T & S (I).

(A) Relative yield

Variable	Bean			Poplar			LSD	Significance		
	WC	RW	UW	WC	RW	UW	(p<0.05)	T (p<)	S (p<)	I (p<)
Root dry wt (g)	0.66 ±0.08	0.85 ±0.09	0.83 ±0.16	0.64 ±0.07	0.38 ±0.04	0.46 ±0.03	0.28	ns	0.01	ns
Stem dry wt (g)	0.74 ±0.01	0.79 ±0.15	0.85 ±0.04	0.56 ±0.11	0.45 ±0.10	0.49 ±0.08	0.28	ns	0.01	ns
Leaf dry wt (g)	0.64 ±0.08	0.83 ±0.14	0.91 ±0.08	0.55 ±0.07	0.45 ±0.10	0.49 ±0.09	0.28	ns	0.01	ns
Fruit dry wt (g)	0.61 ±0.09	0.78 ±0.14	0.84 ±0.09	-	-	-	0.28	ns	ns	ns
Leaf area (dm^2)	0.70 ±0.10	0.86 ±0.14	1.05 ±0.06	0.56 ±0.08	0.51 ±0.11	0.57 ±0.09	0.32	ns	0.01	ns

(B) Relative Yield Total

	Water supply regime			LSD	Significance of effect
	WC	RW	UW	(p<0.05)	T(p<)
Root dry wt (g)	1.30 ±0.09	1.23 ±0.12	1.30 ±0.13	0.46	ns
Stem dry wt (g)	1.29 ±0.20	1.24 ±0.07	1.34 ±0.11	0.32	ns
Leaf dry wt (g)	1.19 ±0.15	1.28 ±0.07	1.40 ±0.13	0.34	ns
Leaf area (dm^2)	1.26 ±0.17	1.37 ±0.05	1.62 ±0.15	0.36	ns

Table 3.4: Competitive performance of bean and poplar, grown in mixed stands in three different soil water supply regimes. Analysis by two way block ANOVA (see Appendix I). Mean of four observations. Watered control (WC); re-watered (RW); unwatered (UW); treatment (T); species (S) and interaction between T & S (I).

Variable	Bean			Poplar			LSD	Significance		
	WC	RW	UW	WC	RW	UW	(p<0.05)	T (p<)	S (p<)	I (p<)
Root dry wt (g)	0.26	0.52	0.46	0.22	-0.30	-0.08	0.39	ns	0.01	0.05
Stem dry wt (g)	0.35	0.41	0.53	0.04	-0.19	-0.06	0.51	ns	0.01	ns
Leaf dry wt (g)	0.20	0.46	0.59	0.06	-0.17	-0.07	0.52	ns	0.01	ns
Leaf area (dm^2)	0.31	0.51	0.73	0.08	-0.05	0.09	0.46	ns	0.01	ns

performance of bean under conditions of freely available water was also greater than that of poplar.

3.3.14 Pre-dawn and mid-day leaf water potential

On day 11 of the second drying cycle, pre-dawn leaf water potential (ψ_{pd}) of both bean and poplar, was significantly lower in treatments RW and UW than in the watered control WC whether grown in monoculture or mixed stands. At all soil water contents, ψ_{pd} was significantly higher in poplar than in bean (Fig. 3.7). The pre-dawn leaf water potential was consistently correlated with soil water content ($r^2 = 0.98$ and 0.96 for bean and poplar, respectively).

At the end of the first drying cycle, a significant reduction of mid-day leaf water potential (ψ_m) was also observed in association with the reduction of soil water content. However, ψ_m was not always consistently related to soil water content and ψ_m of poplar plants was significantly lower than ψ_m of bean plants in mixed stands at the same soil water content (Fig. 3.6). During the period from day 2 to day 14 of the third drying cycle, ψ_m of bean plants, grown either in monoculture or in mixed stands, was significantly lower in treatments RW and UW than in the watered control WC (Fig. 3.8), but in poplar, only on day 14 was ψ_m significantly lower in treatments RW and UW than in WC (Fig. 3.9). However, in both treatments RW and UW there were no substantial changes in ψ_m from day 2 to day 14 when bean and poplar were grown either in monoculture or in mixed stands (Fig. 3.8 and Fig. 3.9). The ψ_m of bean was lower in mixed stands than in the monoculture at all levels of soil water supply but in poplar ψ_m was lower in mixed stands only in the water stress treatments (Fig. 3.6).

3.3.15 Abaxial stomatal conductance

Under conditions of limited soil water supply, the abaxial stomatal conductance of both bean and poplar leaves, grown in both monoculture and mixed stands, was reduced significantly in association with the soil water content on day 12 of the first (Fig. 3.6) and day 11 of the second (Fig. 3.7) drying cycle. Significant reduction of stomatal conductance was also observed in the treatments RW and UW over the period between days 6 and 12 of the third drying cycle in both bean (Fig. 3.8) and poplar leaves (Fig. 3.9). The relationship between stomatal conductance and mid-day leaf water potential was weaker in both bean and poplar plants than the relationship with pre-dawn leaf water potential (Fig. 3.10). Some inconsistencies in the

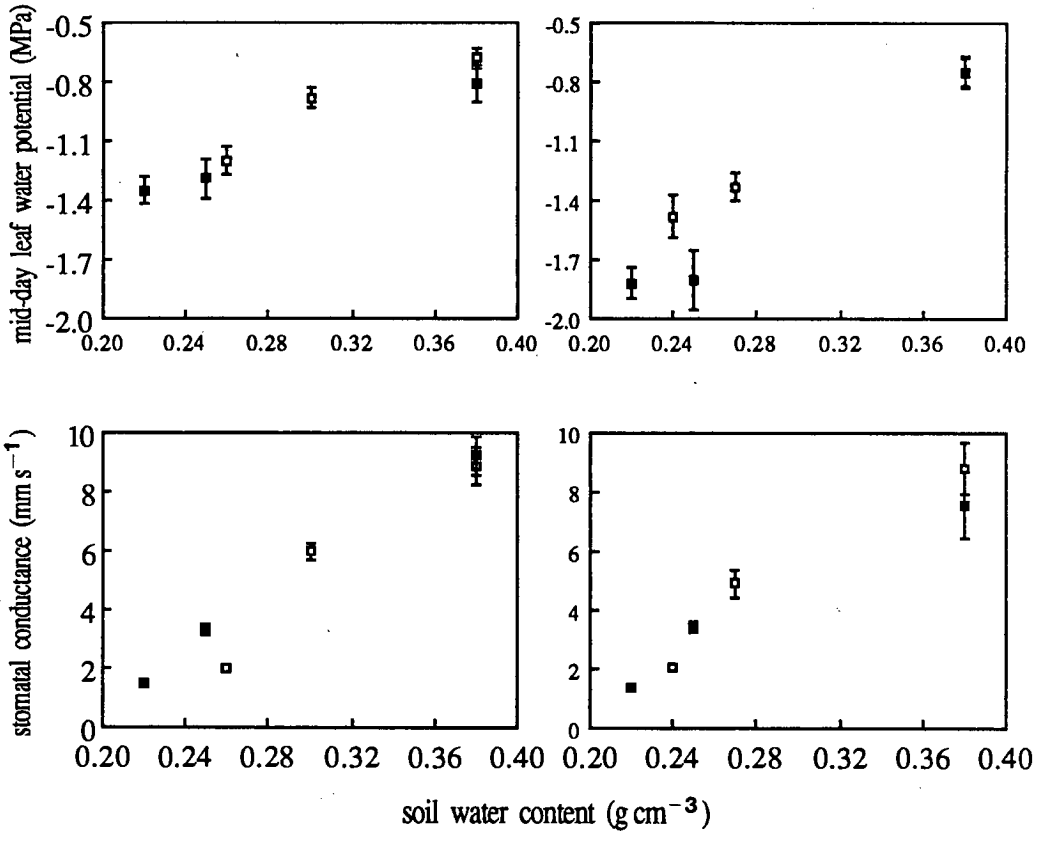

Figure 3.6: Mid-day leaf water potential and abaxial stomatal conductance of bean (left) and poplar (right), grown in monoculture (□) and in mixed stands (■), at various soil water contents in the top 36 cm soil at the end of first drying cycle. Points are means of three and four observations, respectively, ± one standard error of mean.

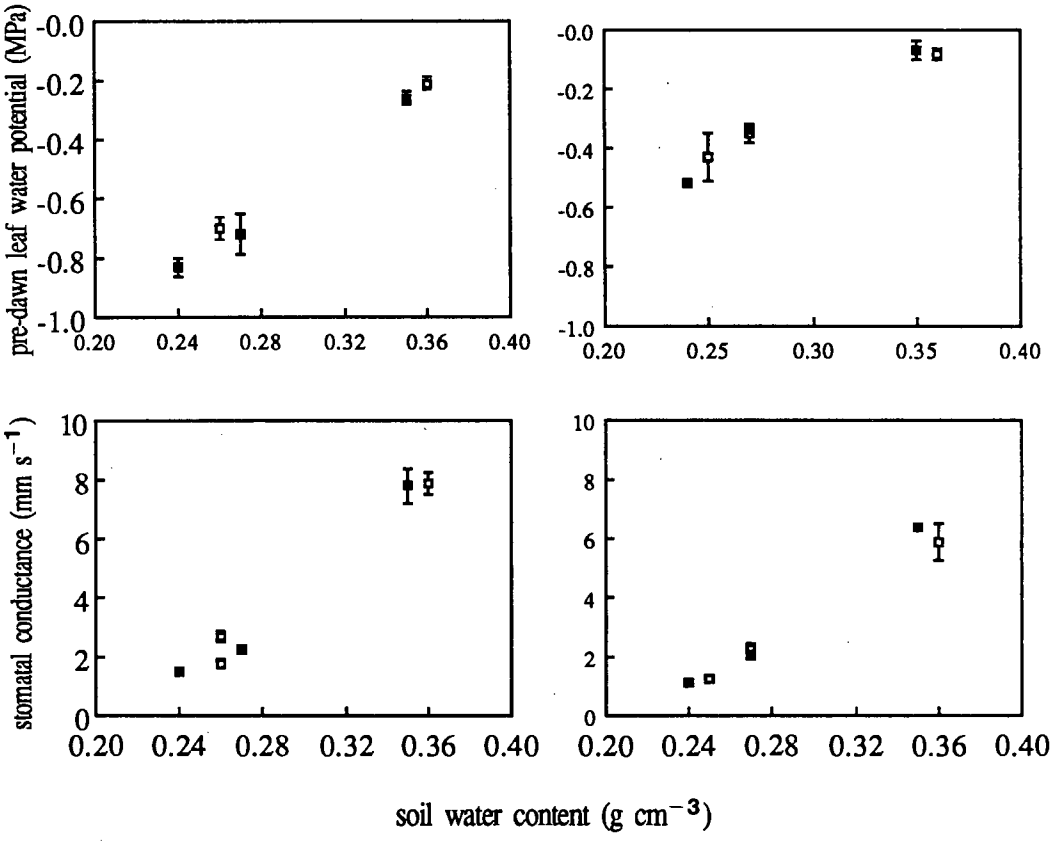

Figure 3.7: Pre-dawn leaf water potential and abaxial stomatal conductance of bean (left) and poplar (right), grown in monoculture (□) and in mixed stands (■), at various water contents of the soil column on day 11 of the second drying cycle. Points are means of three and four observations, respectively, ± one standard error of mean.

relationship between stomatal conductance and mid-day leaf water potential also appeared during the third drying cycle (Figs. 3.8 &3.9).

3.3.16 Nitrogen (N), phosphorus (P) and potassium (K) content

In the watered control treatment WC, there were no significant differences in either N or P concentrations of bean leaves between monoculture (1.94 ± 0.12 and 0.88 ± 0.08) and in mixed stands (1.89 ± 0.08 and 0.82 ± 0.06 % per unit dry wt, respectively). However, leaf potassium concentration was significantly ($p < 0.05$) higher in monoculture (1.99 ± 0.01) than in mixed stands (1.69 ± 0.15) (mean with one standard error, n = 3 in all cases).

There were no significant differences in the concentrations of N, P and K of poplar leaves between monoculture (1.95 ± 0.09, 0.41 ± 0.01 and 1.70 ± 0.06 % per unit dry wt., respectively) and mixed stands (1.94 ± 0.17, 0.44 ± 0.01 and 1.62 ± 0.13, respectively) (mean with one standard error, n = 3 in all cases).

3.4 Discussion

The aim of the present study was to compare the growth and yield responses of an agricultural annual (*Phaseolus vulgaris* L. cv. Argus) and a young tree (*Populus trichocarpa* X *P. deltoides* cv. Raspalje) when grown in mixed stands and in monoculture, in soil columns with three different soil water supply regimes to simulate a model agroforestry situation. Leaf water potential and abaxial stomatal conductance were investigated in order to indicate some of the mechanisms involved in the growth responses.

The significant reduction of mid-day and pre-dawn leaf water potential in both bean and poplar plants towards the end of the first and the second drying cycles, respectively, is an indication of shoot water stress resulting from a shortage of soil water. The abaxial stomatal conductance of both species was also significantly lower. This suggests a relationship between stomatal conductance and leaf water status, as has often been found (e.g. Boyer (1970) in soybean; Jordan and Ritchie (1971) in cotton; Jones and Rawson (1979) in sorghum) although in the present case stomatal conductance was more closely related to pre-dawn leaf water potential (Fig. 3.10). A decline in leaf water potential leading to stomatal closure, previously reported for both

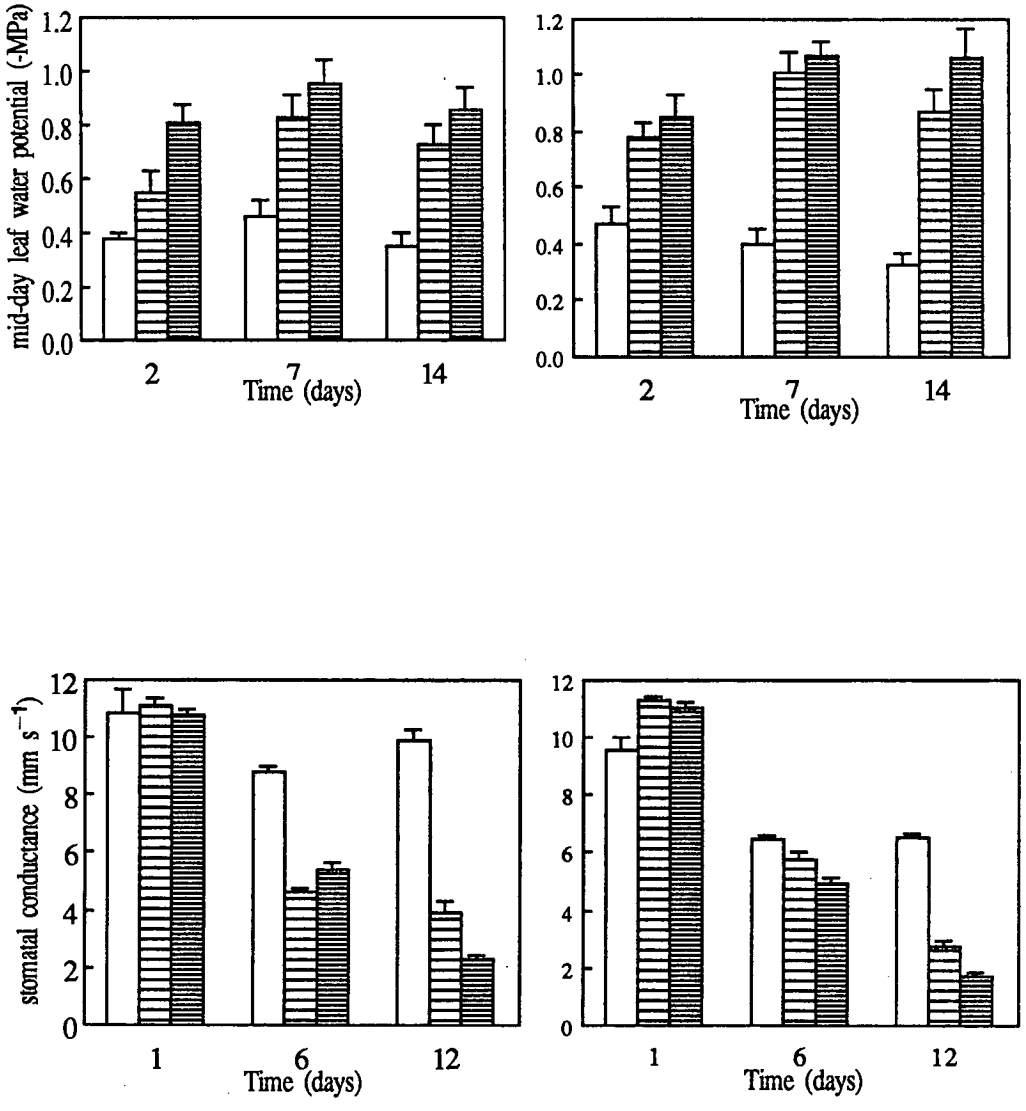

Figure 3.8: Mid-day leaf water potential and abaxial stomatal conductance of bean, grown in monoculture (left) and in mixed stands (right), over the period of third drying cycle in three different soil water supply regimes. (□) watered control WC; (▤) re-watered RW and (▤) unwatered UW. Columns are means of three and four observations, respectively ± one standard error of mean.

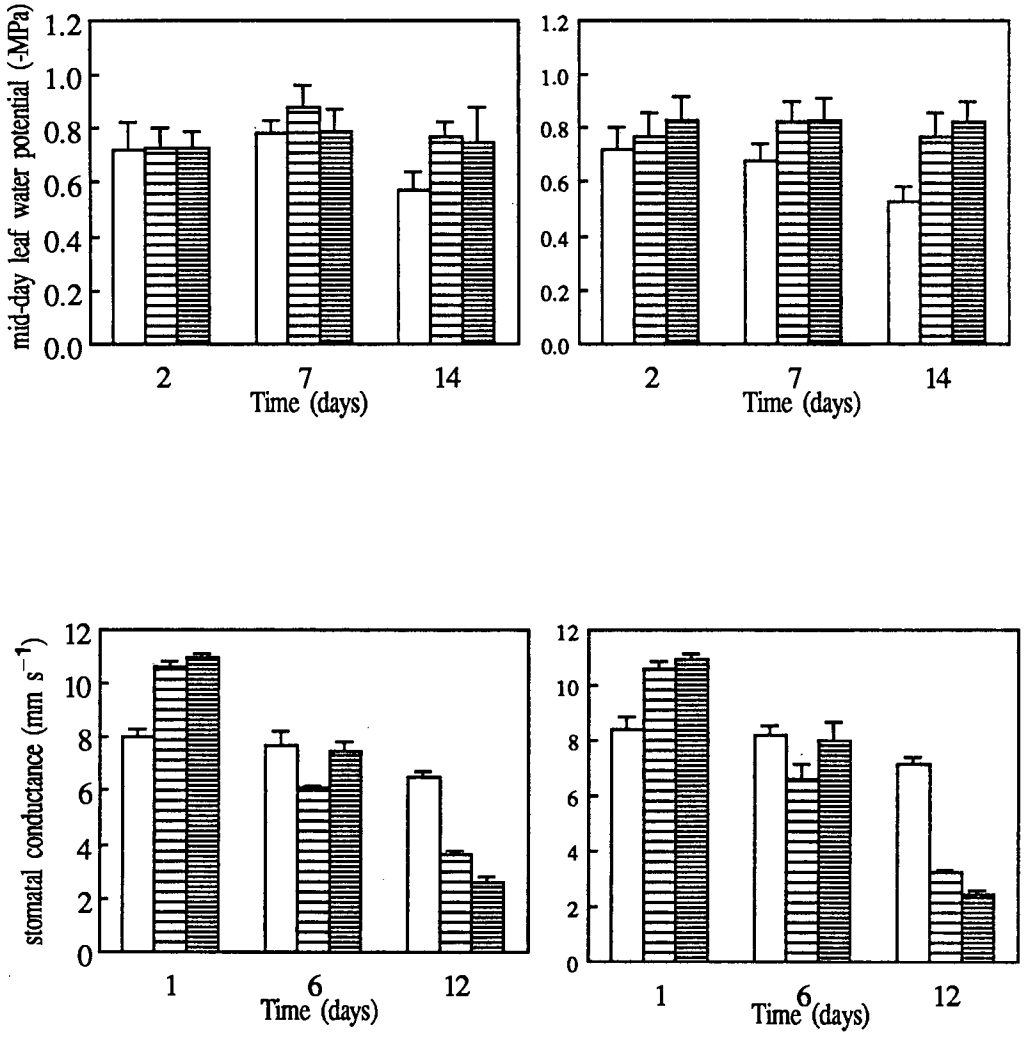

Figure 3.9: Mid-day leaf water potential and abaxial stomatal conductance of poplar, grown in monoculture (left) and in mixed stands (right), over the period of third drying cycle in three different soil water supply regimes. (□) watered control WC; (▤) re-watered RW and (▤) unwatered UW. Columns are means of three and four observations, respectively, ± one standard error of mean.

poplar (Kelliher *et al.*, 1980) and bean (O'Toole *et al.*, 1977; Hegde and Srinivas, 1990), supports the present finding.

However, during the third drying cycle the reduction of stomatal conductance in the water stress treatments occurred without a substantial change of leaf water potential throughout the period, even though the bean plants had a considerably lower leaf water potential than the watered control from the beginning of the cycle. Similarly, with progressive soil drying, stomatal closure also occurred in cow-pea while leaf water potentials were not very different from those of well watered plants (Osonubi, 1985). These observations support the hypothesis proposed by Bates and Hall (1981) that soil drying can influence the transmission of non-hydraulic stimuli from roots to shoots so as to induce stomatal closure. Stomatal closure without change of leaf water status has been reported in several, more recent studies (Turner *et al.*, 1985; Blackman and Davies, 1985; Gollan *et al.*, 1986; Zhang *et al.*, 1987; Masle and Passioura, 1987). An early explanation for observations of this kind was that gradual closure of stomata consequent on soil drying (Mansfield and Davies, 1981) reduces transpiration loss and leaf water potential is maintained (Fischer and Turner, 1978).

The accumulation of solutes in leaf cells during water stress allows a decrease in bulk leaf water potential, together with the partial maintenance of turgor. Solute potential was not measured in this experiment. However, the observations of significantly lower leaf water potentials in bean leaves in treatments RW and UW than in the watered control treatment WC at the beginning of the third drying cycle (Fig. 3.8), although well watered at the end of the second cycle, may be an indication of more solute accumulation during the previous cycle, as found in prestressed leaves of sunflower after seven days of rewatering (Jones and Turner, 1980). However, there was no similar observation in poplar in the present study, in accord with earlier reports (Turk and Hall, 1980; Shackel and Hall,1983).

It is commonly observed that water stress affects leaf growth (see Srinivas Rao and Bhatt, 1988). The significant reduction of leaf growth in bean and poplar leaf with limited soil water supply in the present study is probably related to reduced leaf cell expansion (Cleland, 1971), caused by decline of cell turgor, generally associated with reduced leaf water potential (Boyer, 1968, 1970; Osonubi, 1985). Reduction of cell division rate by water stress can also affect final leaf size (Van Volkenburgh, 1987; Mazzoleni and Dickmann, 1988), and change of cell wall properties under water stress may also control leaf growth (Rhizopoulou, 1990). Reduced leaf water potential,

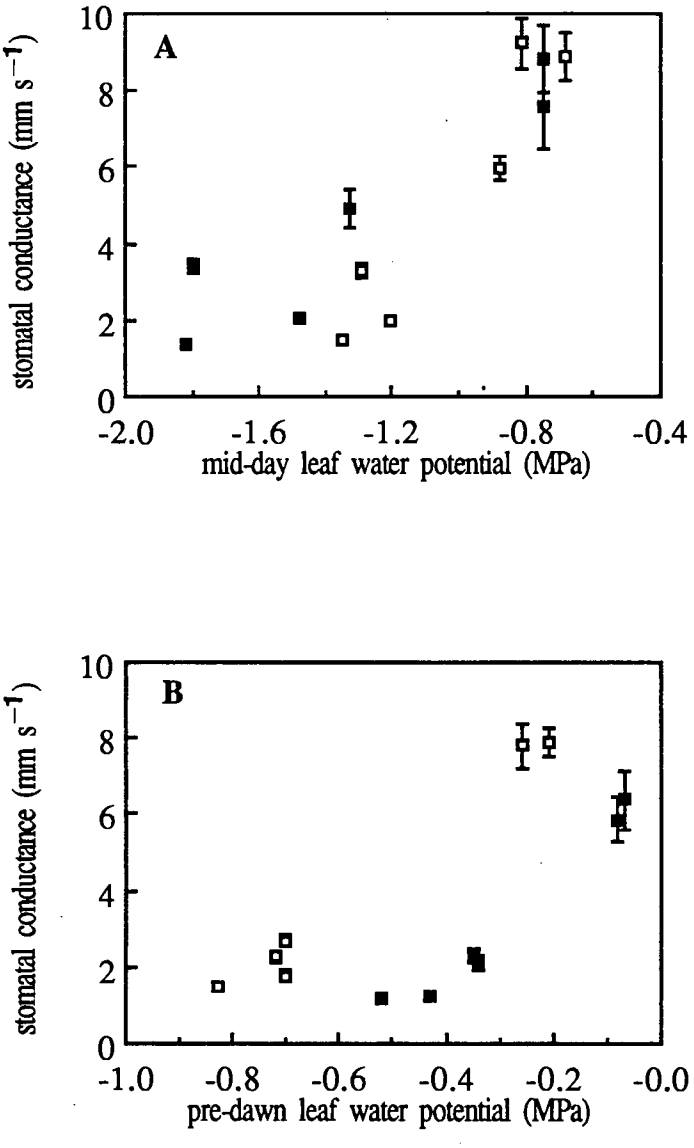

Figure 3.10: Abaxial stomatal conductance of bean (□) and poplar (■) at various mid-day leaf water potentials (A) and pre-dawn leaf water potentials (B) at the end of the first and 11th day of the second drying cycle, respectively. Points are means of four observations ± one standard error of mean.

resulting from limited water supply, can decrease net photosynthesis, so leaf growth is inhibited, as reported in sorghum (Shearman *et al.*, 1972), soybean (Beardsell *et al.*, 1973) and French bean (O'Toole *et al.*, 1977). Closure of stomata in response to limited soil water supply, inhibits carbon dioxide exchange with the result that reduced supplies of assimilate are available for allocation to the growing leaves, and as a result, leaf growth is affected, as reported in wheat (Masle and Passioura, 1987). During water stress, a close correlation between photosynthesis and stomatal conductance has been reported in seedlings of many tree species, e.g. *Eucalyptus globulus* (Metcalfe *et al.*, 1989) and red spruce (Seiler and Cazell, 1990). However, during the second cycle, the maintenance of leaf expansion in bean plants, particularly in mixed stands experiencing severe water stress (Fig. 3.5A), suggests that turgor has been maintained in the cells of expanding leaves. Increasing solute accumulation in leaf cells (e.g. Hsiao *et al.*, 1976) or limited reduction of leaf water potential because of maximum stomatal closure (Rhizopoulou and Davies, 1991) can contribute in this respect.

The total leaf area of stressed bean and poplar plants was reduced because the development of final leaf size was affected by water stress (Fig. 3.5). However, decline in leaf appearance rate (Metcalfe *et al.*, 1990) could also be attributed to such reduction although leaf appearance was not measured in this experiment. Surprisingly, there was no difference in total leaf area between water-stressed and watered control plants of bean, when grown in the mixed stands (Table 3.1A). Possibly, bean competed better than poplar when the water supply was limited and utilized available water and nutrients at the expense of the poplar plants. A reduction in leaf development of watered control plants in the mixed stands resulting from competition for water might be another cause: the lower leaf water potential of bean in mixed stands than in monoculture in the watered control (Fig. 3.6), could be an indication of this. Higher leaf water potentials in *Encelia farinosa* without neighbours than with neighbours (Ehleringer, 1984) supports this argument.

The leaf dry weight of bean plants was unaffected by water stress, although leaf area declined, so that the leaf area per unit leaf dry weight (SLA) declined, probably because increase in leaf cell wall content and vascular tissue (e.g. Pitman *et al.*, 1983) makes the leaf thicker. However, the SLA of poplar was unaffected and even increased in the water-stressed mixed stands, as reported by Bachelard (1986) for some *Eucalyptus* species. The LWR (leaf weight ratio) of poplar remained unchanged in all three water supply regimes, possibly because of the close relationship between

leaf development and carbon fixation (Michael *et al.*, 1988), whereas in severely water-stressed bean plants the LWR increased: because of reduced leaf expansion leaves become thicker with possible increase in cell wall content and more solute accumulation in leaf cells.

Stem elongation was reduced in bean and poplar in conditions of limited water supply, in agreement with observations reported elsewhere (e.g. Turner *et al.*, 1985; Flower and Ludlow,1986; Kelliher *et al.*, 1980), possibly because of reduced cell expansion or shortage of assimilate supply (Gollan *et al.*, 1985).

As reported by Sharp and Davies (1985), root biomass production of poplar plants was reduced significantly by soil water stress, whereas bean root biomass was unaffected, indicating maintained assimilate allocation to bean roots in that treatment (see also Hsiao and Acevedo, 1974; Sharp and Davies, 1979). With limited soil water, although total root development of plants is reduced substantially, roots often grow deeper into the subsoil (Sharp and Davies,1985). The distribution of significant amounts of bean and poplar feeding roots towards the deeper regions of the soil column in the treatments RW and UW, relative to WC plants (Fig. 3.3), is in agreement with this. The possible higher concentration of abscisic acid in roots of water-stressed plants may act to promote root growth (Watts *et al.*, 1981) and thereby enable roots to penetrate deeper into the sub-soil. Soil water extraction by the bean and poplar plants was related to their root distribution, with soil moisture depletion by bean predominantly in the top 36 cm and by poplar throughout the soil column. In the water stress treatments, the roots of bean were significantly restricted to the top 36 cm of the soil column, whereas the poplar roots were distributed uniformly down to the bottom of the soil column. This can be attributed to the genetically determined shallow and deep rooting characteristics of annual crops (Blum, 1974) and trees (Osonubi and Davies, 1981), respectively.

The lower leaf water potentials and stomatal conductances of both species in mixed stands than in monocultures in the water stress treatments (Fig. 3.6) can be related to competition for water (Ehleringer, 1984). Since plant water potential before dawn is likely to be close to equilibrium with soil water potential (Fonteyn and Mahall, 1981), the observation of significantly lower pre-dawn leaf water potential in bean plants than in poplar plants in mixed stands (Fig. 3.7), indicates more soil water stress in the bean plants, presumably because of their limited pattern of root exploration of the soil profile: the root system of bean was only able to explore the top soil layer, which was

relatively drier, whereas poplar roots were able to explore further down the soil column as well. Thus the tree species have a complementary rooting pattern with the result that the relative yield total (RYT) exceeded 1.00 (Table 3.4B). This is an agreement with results described by Remison and Snaydon (1980) who stated that, if RYT is greater than 1.00, the two component species are not completely competing for the same resources.

However, restriction of soil water extraction to the top 36 cm depth of the soil column by bean indicates competition for water with poplar in the upper soil layers that must have been greater during the period of poplar cutting establishment, when soil water extraction by poplar was also restricted to the uppermost 36 cm. There was comparatively more soil moisture depletion in the top 36 cm of the soil column by the mixed stands of bean and poplar than by the monocultures and this also indicates competition for water in the uppermost soil layer. The growth suppression of *Acacia tortiles* (tree) for the first three years by *Cenchrus ciliaris* (grass) as a result of competition for water in the upper soil layer (Muthana *et al.*, 1985) followed a similar pattern: competition between woody legumes and yam (*Dioscarea alata* cv. Brazo Fuerte) for water in the upper soil layer has also been reported (Budelman, 1990). The index of competition (Rhodes, 1981) showed that poplar growth was suppressed significantly by competition under limited soil water supply (Table 3.4). On the other hand the relative yield of bean in mixed stands was enhanced (Table 3.3). As a result, the contribution of bean plants to RYT was significantly more than the contribution of poplar. Probably because of the significantly smaller root system developed by poplar under water stress than by bean, poplar plants were less able to compete for soil water. This is similar to the situation studied by Thomas (1984) in clover and ryegrass, where clover had the less well-developed root system.

Although the yield of each species in the mixed stands was competitively enhanced in the watered controls, the total yield of each species was reduced significantly compared with the monocultures, indicating possible competition for water and nutrients. Competition for light was not likely to have been effective in this experiment because light can penetrate both from above and from the sides in pot-grown plants (see also Thomas, 1984), especially in wide spacings, as here. Nutrient availability in the soil depends on soil water supply, as regular water supply to the soil makes nutrients more available to plants (Viets, 1972). In the watered control of the present study, the nutrients in the soil were freely available to the component species in the mixed stands, and both started to grow well, but possibly competition for nutrients

developed later. However, there were no significant reductions in concentrations of N, P and K in the foliage of bean or poplar in the mixed stands relative to the monocultures, except in the case of K in bean where the situation was complicated by luxury uptake. Consequently, it was felt that competition for water, rather than for nutrients, was the main cause of changes in growth.

With limited soil water supply, the yield of bean plants in mixed stands was unaffected, although in monoculture yield was significantly reduced. However, poplar yield was affected in mixed stands as in the monoculture, although yield in treatment UW was statistically similar to yield in treatment RW in the mixed stands. These responses could reasonably suggest good performance by both bean and poplar in an agroforestry system experiencing severe water shortage. The results reported here suggest that the differential growth responses of annuals and young trees to soil drying could provide basic information on which to base the selection of compatible and complementary species for a successful agroforestry plantation in a water shortage area. Stomatal conductance of both species was more strongly related to pre-dawn leaf water potential (a measure of soil water status) than to mid-day leaf water potential, in support of the hypothesis yet to be investigated that shoots may respond to soil water status independently of leaf water status. Earlier responses of shoots to soil drying could maintain sustained productivity of plants in a water shortage area before major, damaging effects of water stress develop, and this may be considered as an important mechanism in agroforestry situations.

CHAPTER 4

Growth and Stomatal Response of Bean and Poplar to Soil Drying in Relation to Shoot Water Supply

4.1 Introduction

It is widely accepted that the growth and stomatal conductance of plants are affected by limited soil water supply. Shoot water relations and a possible secondary sensing mechanism by the roots involved could have an effect either independently or together. As soil dries, the soil and root water potential falls, leading to decline in leaf water potential and turgor potential, and to the closure of stomata (e.g. Turner, 1974). Growth is sensitive to water deficit and can also be regulated by turgor potential (Kramer, 1983). Decline in leaf water or turogor potential, as a result of soil drying, leading to stomatal closure has been observed in both poplar (Kelliher *et al.* 1980) and in bean (O,Toole *et al.*, 1977; Hegde and Srinivas, 1990) as reported in Chapter 3.

However, careful investigation of plant response to soil drying reveals that stomatal closure can occur before any change in shoot water status is detected, for example as reported in a field study of cow-pea (Bates and Hall, 1981). A similar response has also been observed by Blackman and Davies (1985) in maize plants, in an experiment with a root system split between two different pots, one of which was subjected to drying. In these experiments stomatal closure during soil drying probably reduced transpiration loss so that leaf water potential was maintained and may even have increased. Jones (1985) for example reported higher leaf water potentials in field grown apple seedlings during soil drying than in well-watered plants. Similarly when turgor was maintained by applying pressure to the root system during soil drying a reduction of leaf growth was also found (Passioura, 1988). Increasing evidence of this kind indicates that stomatal conductance and growth are controlled by soil water status independently of shoot water status (e.g. Turner *et al.* 1985; Gollan *et al.* 1986; Zhang *et al.* 1987).

In these circumstances, signals from roots must be considered in the regulation of shoot response, at least in maize (Blackman and Davies, 1985; Davies *et al.* 1986; Saab

and Sharp, 1989), sunflower (Zhang and Davies, 1989b)and apple clones (Gowing *et al.* 1990). Research has shown that the signal is chemical and is apparently ABA (Zhang *et al.* 1987, Zhang and Davies, 1989a &b). It is presumed that dehydrating roots produce ABA which is transported to the leaves through xylem flow resulting in stomatal closure (Davies and Zhang, 1991). However, Aspinall (1980) questioned the importance of root signals because shoot water potential falls more rapidly than root water potential. As leaves obtain water from a well-watered soil along a water potential gradient and transpire continuously, sudden onset of soil drying could immediately induce hydraulic tension in the xylem, resulting in a decline in leaf water potential. Any small water deficit in leaf caused by drought can also promote ABA production in leaves (Hartung *et al.* 1990). Generally during drought water deficits develop first in older leaves which produce more ABA that can be transported to younger leaves (Cornish and Zeevaart, 1984), resulting in increased accumulation of ABA in young leaves. Thus the hydraulic influence of drying soil on shoot growth and stomata can not be ruled out.

The present experiments were designed to investigate the growth and stomatal response of bean and poplar during soil drying in two separate experiments. Plants were grown in specially designed pots with horizontally divided root systems. The root systems present in the upper pots were subjected to soil drying while water was supplied to the root systems present in the bottom pots with a view to maintaining a continuous shoot water supply. Leaf water relations and ABA concentrations in roots and leaves were determined in order to separate the hydraulic and non-hydraulic influence of drying soil on growth and stomata.

4.2 Materials and Methods

4.2.1 Plant materials and design of the experiments

Cuttings of poplar and seeds of bean were sown in small pots containing vermiculite and perlite to raise rooted cuttings and seedlings, respectively. Pre-rooted cuttings of poplar (8 weeks old) and seedlings of bean (at two leaf stage) were transplanted into separate pots containing a compost (50% loam, 25% sand and 25% peat). The pots were specially designed with one pot above the other . A number of large holes were made in the bottom of the upper pot so that the roots of the plants could pass through easily to the lower pot. In the case of poplar the upper and lower pots contained

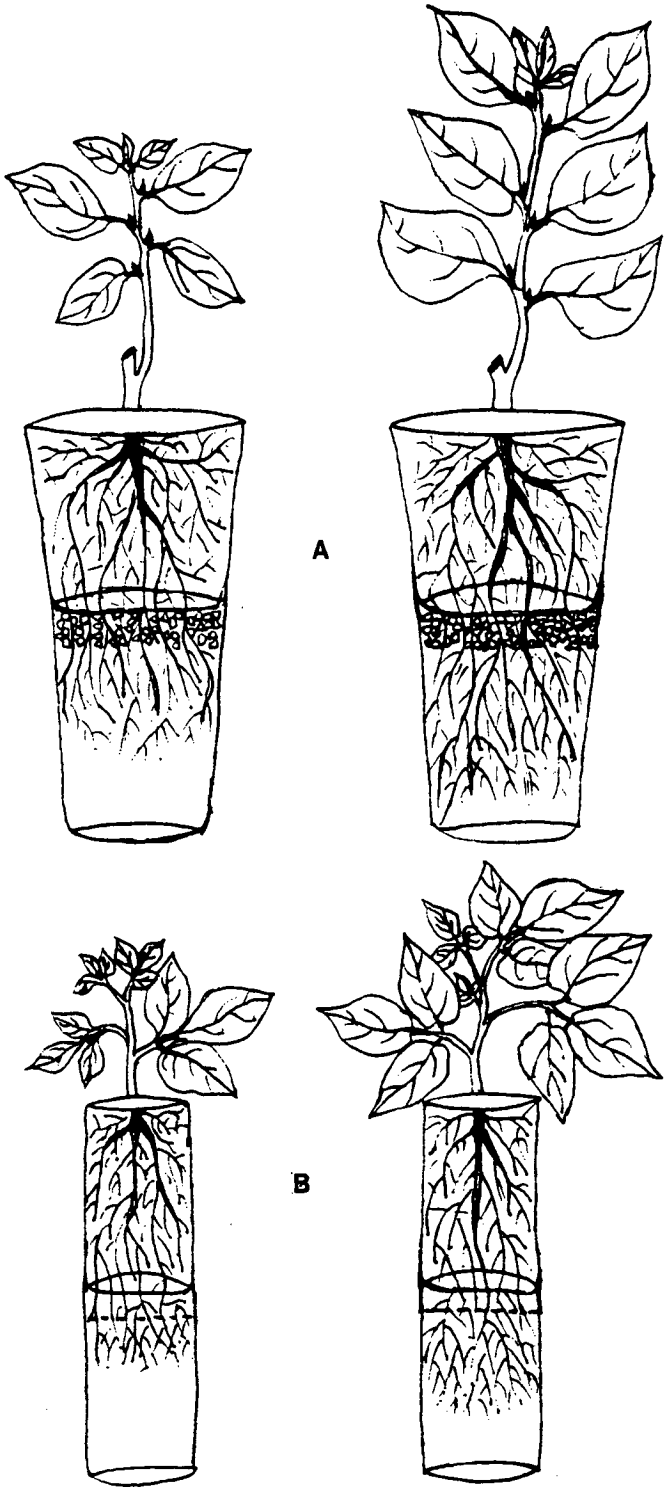

Figure 4.1: Diagramatic sketch of specially designed pots with soil columns supporting poplar (A) and bean (B) plants, showing root system distribution on the day the experiment started (left) and ended (right).

18 cm and 16 cm diameter soil columns with volumes of *ca* 3500 cm^3 and *ca* 3000 cm^3, respectively. In between the two soil columns there was 4 cm of small gravel so as to prevent the capillary movement of water from the lower soil column to the upper one. In the case of bean, both upper and lower pots contained soil columns of the same volume of *ca* 1000 cm^3 and in between the two soil columns there was a 2 cm humid gap. All the plants were watered regularly until they were established and the roots of the plants had access to the lower soil column before starting the treatment. The treatment was started 8 weeks after transfer in the case of the poplar and in the case of the bean when the seedlings had the first fully expanded trifoliate leaf. At that time poplar roots occupied a significant volume of the lower soil column (Fig. 4.1 A left) but bean roots occupied only a very limited volume (Fig. 4.1 B left). The layout of the plants in both experiments was in a completely randomized design. From the day each experiment started (day 1), half the plants were watered every evening from above (control) and the other half (the treatment) received no water from above, but in both cases the lower soil column was kept moist by water from below. The idea was to maintain the supply of water to the shoot by roots in the lower moist soil column. The experiments for poplar and bean were done separately during the months of June and July, 1990, respectively, in the greenhouse. The period of the drying cycle was 17 and 11 days, respectively. After the end of these periods stomatal conductance, leaf water potential and leaf expansion of poplar and stomatal conductance of bean were measured for a few days although no water was added to the upper soil column of the treatment, when the roots of treated plants had enough access to lower moist soil column.

4.2.2 Microclimate

The microclimate was uncontrolled in the greenhouse. To show the variation of conditions during the experimental periods, the photosynthetic photon flux density incident inside the greenhouse was recorded using a calibrated quantum sensor (LI - 190 SB Li Cor Inc., Lincoln, USA). The temperature of air entering the greenhouse was also recorded using a copper constantan thermocouple (British standard BIO BS 1843 Type T, T. C. Ltd., Uxbridge, UK). A data logger (Deltalogger, Delta-T Devices Co. Ltd., Cambridge, UK) was used to obtain hourly averages of readings that were made at 10 minute intervals. Because some data were missing from the data logger, approximate values of radiation and temperature for the missing period (last 7 days in the poplar experiment and first 5 days in the bean experiment) were obtained from charts of the Department of Meteorology, University of Edinburgh. The

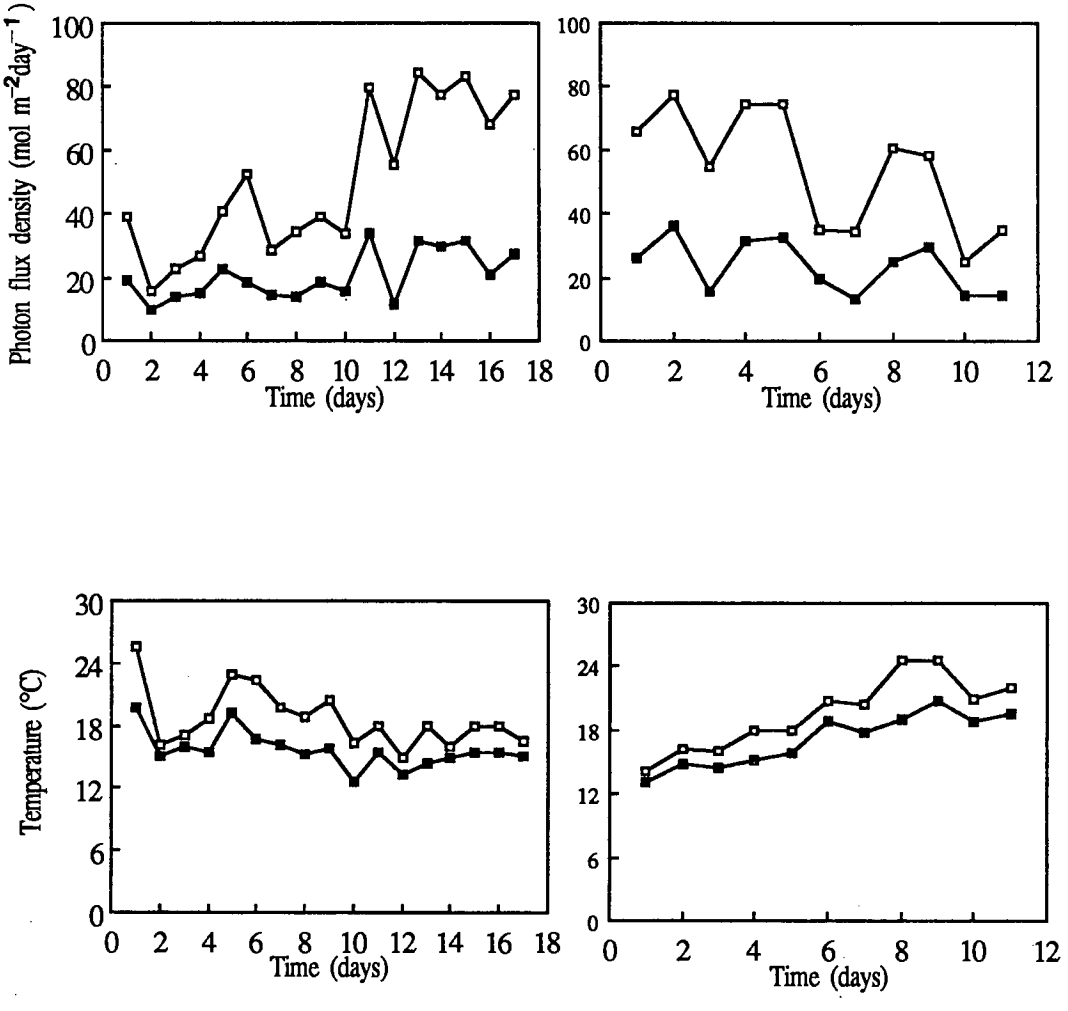

Figure 4.2: Variation in photosynthetic photon flux density and temperature during the photoperiods of the drying cycles of 17 and 11 days of the experiment of poplar (left) and bean (right). ■ mean and □ maximum.

measurements during the 14 hours of photoperiod are presented as mean and maximum values (Fig. 4.2).

4.2.3 Measurement of soil water content

The entire soil column was removed from the pot and samples were collected throughout, except on day 4 and 13 of the poplar experiment, when a metallic corer (15 mm diameter) was used. Four replicate measurements were made from the upper soil column of each group (control and treatment) after every three days in the case of poplar and after every two or three days in the case of bean. Fresh and oven dry (at 90 °C for 48 hours) weights of the samples were recorded and water contents of the soil were calculated gravimetrically.

4.2.4 Stomatal conductance

Measurements of abaxial stomatal conductance were made using a steady-state null-balance porometer (LI 1600, Li-Cor Inc. Lincoln, Nebraska, USA) for poplar and a transient diffusion porometer (Mark II, Delta-T Devices Co. Ltd, Cambridge, UK) for bean. The most recent fully expanded leaf, as well as an expanding leaf, on each plant were measured and the mean of these measurements was recorded as the stomatal conductance of each plant. Eight plants of control and treatment of each species were measured after 5-6 hours of photoperiod on the day of measurement of soil water content.

4.2.5 Leaf water relations

On finishing the measurement of stomatal conductance, the leaf water potential of the most recent fully expanded leaf was measured on the day of measurement of stomatal conductance using a pressure chamber. In poplar the 5th or 6th leaf from the stem apex and in bean the terminal leaflet of the1st, 2nd or 3rd trifoliate leaf (depending on the day of measurement) was measured. Leaf lamina portions were taken from the leaf used for the measurement of leaf water potential and frozen in liquid nitrogen. Frozen leaf samples were thawed and pressed in a disposable syringe to collect sap extract. Osmotic potential of 10 mm^3 sap extract was measured using a vapour pressure osmometer (VPO, Wescor model 5100 C, Wescor Inc., England). The turgor potential was calculated by difference between leaf water potential and osmotic potential.

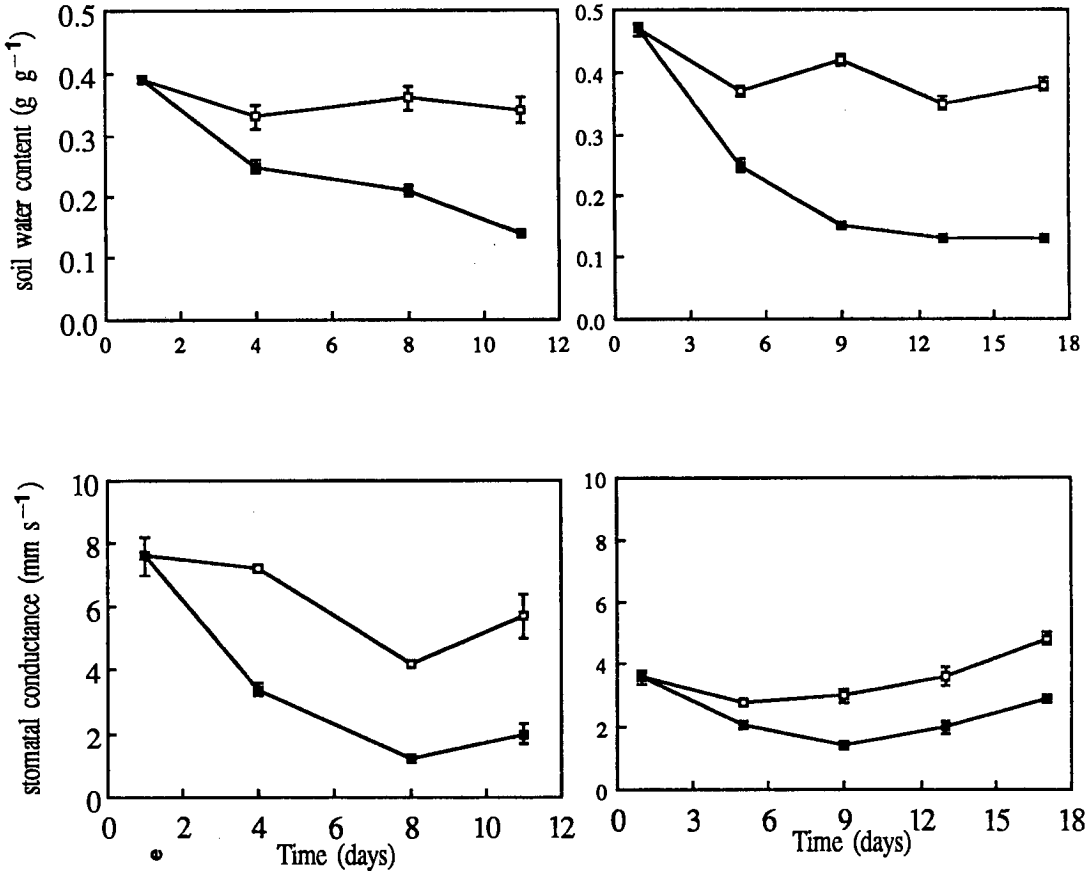

Figure 4.3: Change in soil water content of the upper soil column and abaxial stomatal conductance as a function of time for bean (left) and poplar (right) after water was withheld from day 1. □ well watered and ■ unwatered. Points are means of four and eight measurements, respectively ± one standard error of the mean.

Measurements were made on six leaves of the control and treatment of each species using one leaf from each plant.

4.2.6 Expansion in leaf/leaflet area

Growing leaves and terminal leaflets of poplar and bean, respectively, were selected for the measurement. The length and width of poplar leaves were measured and later converted into areas (see Section 3.2.3). The area of the terminal leaflet of bean was measured directly using a portable leaf area meter (CI 201, Portable Area Meter, CID, Inc, Moscow, USA). Measurements were made on six plants of control and treatment of poplar and bean on days 1, 5, 9, 13 and 17 and on days 1, 3, 5, 7, and 9 of the drying cycles, respectively. The same leaves/leaflets were measured throughout the period. The mean initial size for control and treatment plants was 15.27 ± 1.46 cm^2 and 15.20 ± 1.32 cm^2, respectively in the case of poplar and in the case of bean 13.21 ± 0.89 cm^2 and 13.46 ± 0.71 cm^2, respectively. Expansion of leaf/leaflet area is presented (Fig. 4.8 and 4.9).

4.2.7 Increase in stem length

The stem length of poplar and the length of a typical growing shoot, developed from a node of the main stem of bean were measured. Measurements were made on six plants of control and treatment of each species on the day of measurement of leaf area. The same stems/growing shoots were measured throughout the period. Increase in stem length was calculated by subtracting the initial length from lengths increased subsequently.

4.2.8 Specific leaf area

Approximately 2 cm^2 of lamina was taken from three leaves (expanding, most recent fully expanded and older leaf) of each plant. The area (measured by leaf area meter LI 3100, Li Cor Inc., Lincoln, USA) and oven dry (at 90 °C for 48 hours) weight of these were recorded and specific leaf area (SLA) derived as leaf area/leaf dry weight. Measurements were made on six plants of control and treatment of each species on the day of measurement of soil water content.

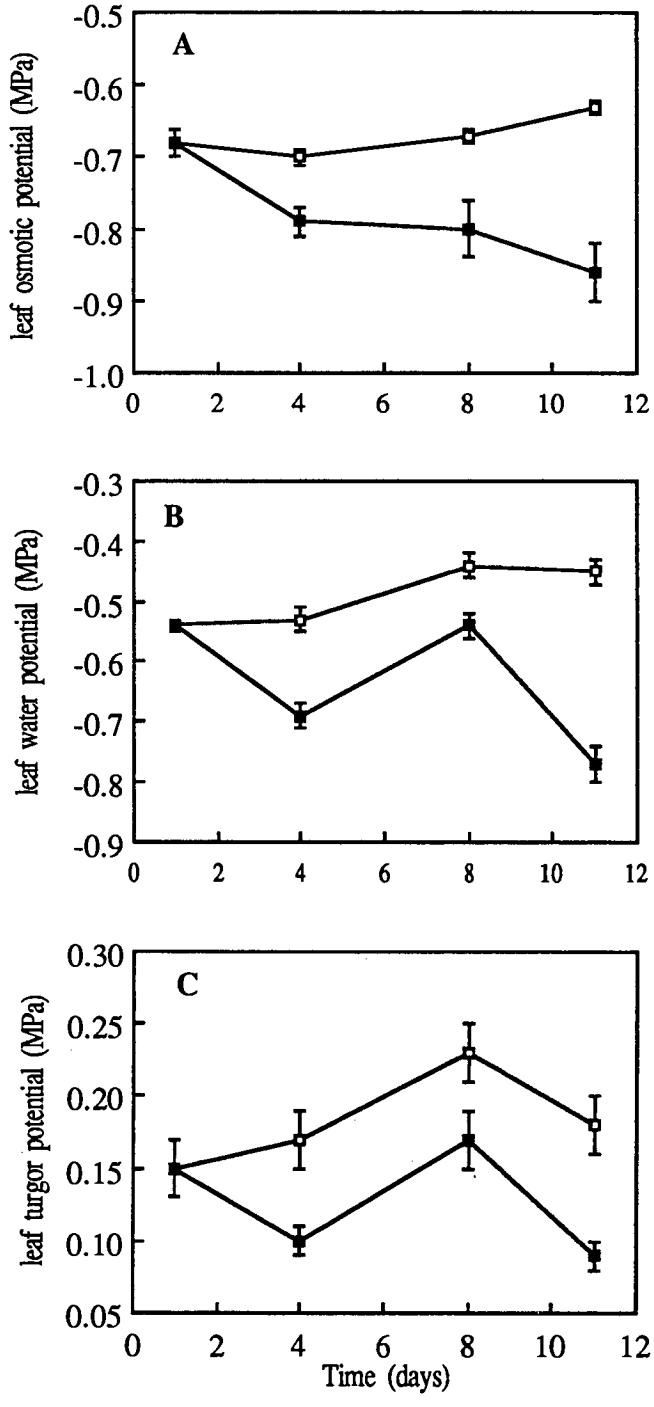

Figure 4.4: Osmotic potential (A), bulk water potential (B) and turgor potential (C) of fully expanded bean leaves as a function of time after water was withheld from day 1. □ well watered and ■ unwatered. Points are means of six measurements ± one standard error of the mean.

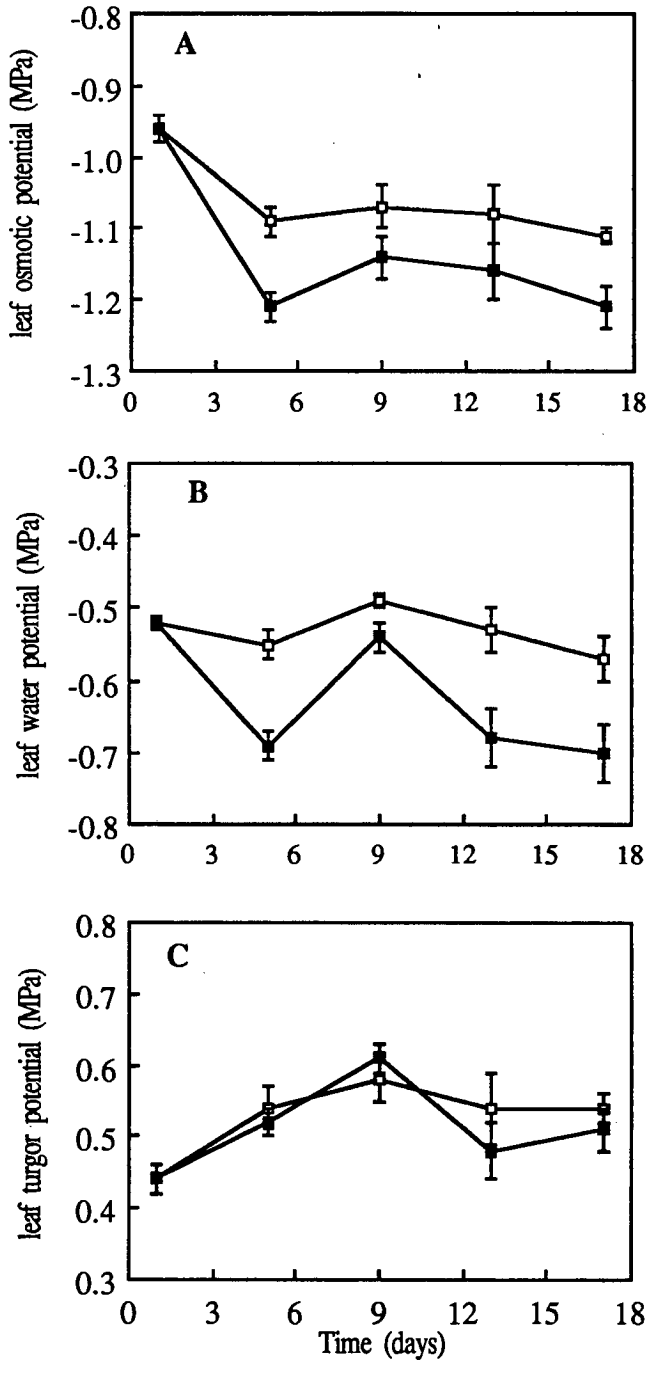

Figure 4.5: Osmotic potential (A), water potential (B) and turgor potential (C) of fully expanded poplar leaves as a function of time after water was withheld from day 1. □ well watered and ■ unwatered. Points are means of six measurements ± one standard error of the mean.

4.2.9 Measurements of leaf and root abscisic acid (ABA)

Lamina portions from the leaf margin were taken from the leaf used for the measurement of leaf water potential. The whole soil column was removed from the pot to expose the root system and immediately the root tips were collected. The root tips of watered plants were washed quickly and blotted immediately, but the root tips of unwatered plants were cleaned only. Samples of leaf and root were wrapped in aluminium foil and frozen in liquid nitrogen. The period required for the collection of each leaf and root sample was 3-4 and 5-6 minutes, respectively. These frozen samples were stored in a freezer (-80 °C) and later used in the determination of abscisic acid (ABA) concentration using the radioimmunoassy (RIA) protocol (Appendix II), described by Quarrie *et al.* (1988). Measurements were made on four replicates of control and treatment of each species on the day of soil moisture content measurement. Leaf and root samples from which ABA was extracted overnight were oven dried (at 90 °C) later to get the dry weight of each sample.The results are expressed as ng of ABA per g of dry weight of sample.

4.2.10 Data analysis

Means and standard errors of the mean of each variable on each occasion were calculated and presented in the form of graphs. Where the error bar of control and treatment means overlapped or were very close to each other, the paired data were tested by Students t-test, using the STATVIEW package.

4.3 Results

4.3.1 Microclimate

During the 17 day drying period of the poplar experiment (during the daylight hours) the ranges of mean daily and maximum photosynthetic photon flux density (PPFD) were 11-33 and 15-84 mol m^{-2} day^{-1}, respectively. The mean daily temperature range was 13-20 °C with maximum temperature in the range 15-26 °C (Fig. 4.2 left). During the 11 day drying period of the bean experiment the mean and maximum PPFD were 13-36 and 24-77 mol m^{-2} day^{-1} with temperatures of 13-21 °C and 14-25 °C, respectively (Fig. 4.2 right).

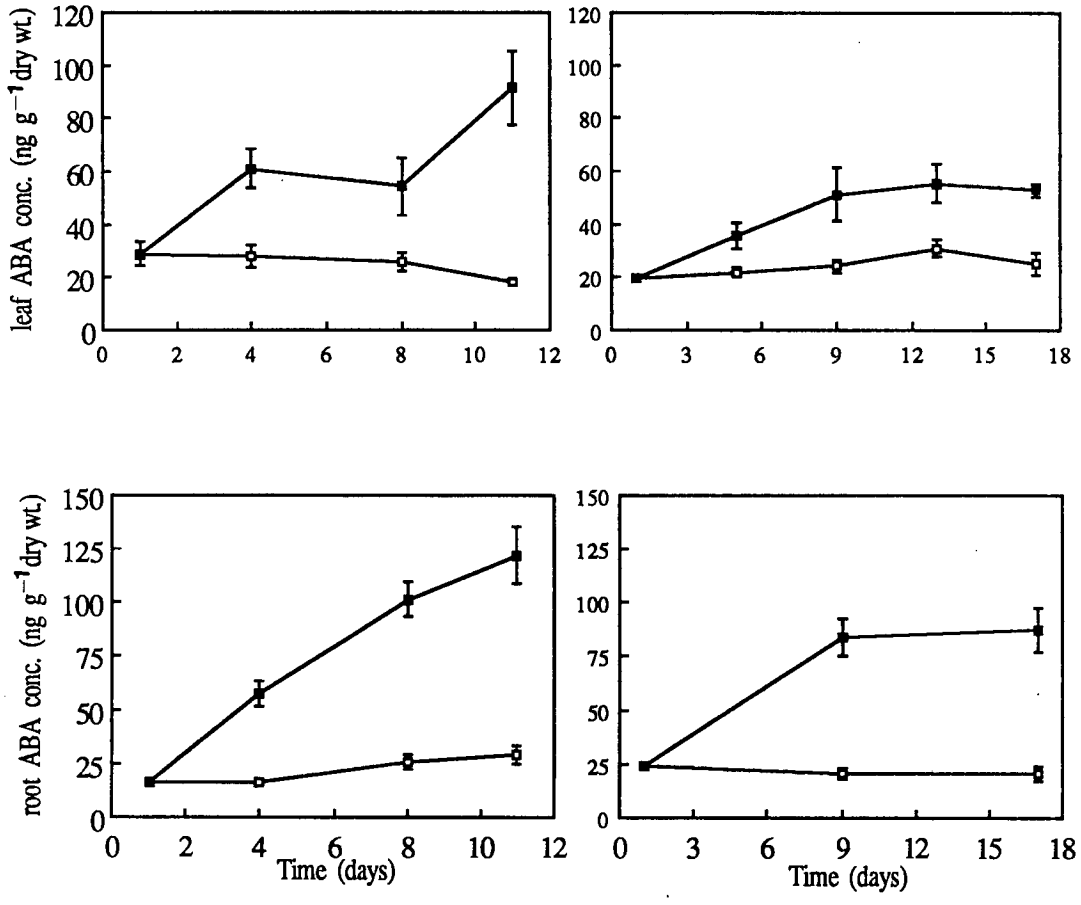

Figure 4.6: Change of abscisic acid concentration in leaves and roots of bean (left) and poplar (right) as a function of time after water was withheld from day 1. □ well watered and ■ unwatered. Points are means of four measurements ± one standard error of the mean.

4.3.2 Soil water content

Withholding of water, together with rapid water loss from the soil column by evapo-transpiration, resulted in significant reduction of soil water content in the upper (unwatered) soil column with time (Fig. 4.3). After day 9, the decline of soil water was insignificant in the soil column with poplar plants (Fig. 4.3 right).

4.3.3 Abaxial stomatal conductance

With progressive drying of the top soil column there was a gradual decline of stomatal conductance which was significant in both bean and poplar, although some of their roots had access to the lower moist soil layer. However, stomatal conductance appeared to increase gradually after day 8 and 9 of the drying period, respectively (Fig. 4.3). The stomatal conductances of treated plants recovered to those of control plants on day 14 and 22 of the drying period in bean (data not shown) and poplar (Fig. 4.11A), respectively, although there was a large amount of roots in the upper dry soil column. At that time roots also occupied a significant volume of the lower moist soil column (Fig. 4.1 right). With both species there were linear relationships between stomatal conductance and soil water content and leaf ABA but not with bulk leaf water potential (Fig. 4.7 and 4.8). In both cases the relationship was stronger with soil water than with leaf ABA.

4.3.4 Leaf water relations

Like stomatal conductance, the bulk leaf water potential in both bean and poplar also declined during the drying period along with the initial (on day 4 and 5, respectively) decline of soil water content. But with further decline of soil water, although the stomatal conductance declined simultaneously, the leaf water potential increased (on day 8 and 9, respectively) close to that of the control plants (Fig. 4.4B and 4.5B, respectively). Later, the leaf water potential of the treatment plants declined again while the stomatal conductance appeared to increase gradually. However, the turgor potential of the poplar treatment plants remained similar to that of the control plants throughout the period (Fig. 4.5C), in contrast to the bean plants where turgor potential significantly decreased in the treatment plants (Fig. 4.4C). On day 22 of the poplar experiment, although the stomatal conductance of treatment plants was significantly higher than that of control plants, the bulk leaf water potential was significantly lower (Fig. 4.11).

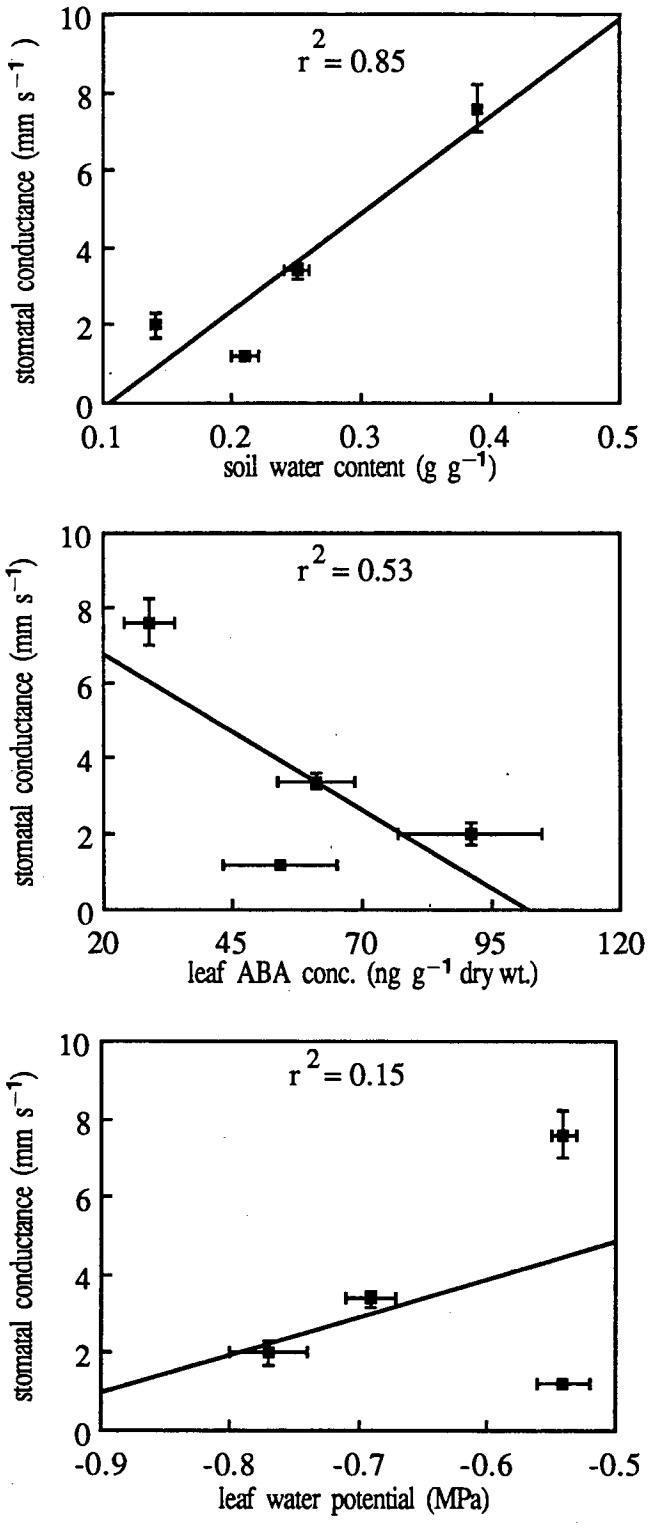

Figure 4.7: Linear relationships between abaxial stomatal conductance (n = 8) and soil water content (n = 4), leaf ABA concentration (n = 4) and leaf water potential (n = 6) in bean plants. Points are means ± one standard error of the mean. Data were collected over a time course of soil drying, sampling the plants at 5-7 hours into the photoperiod of each day.

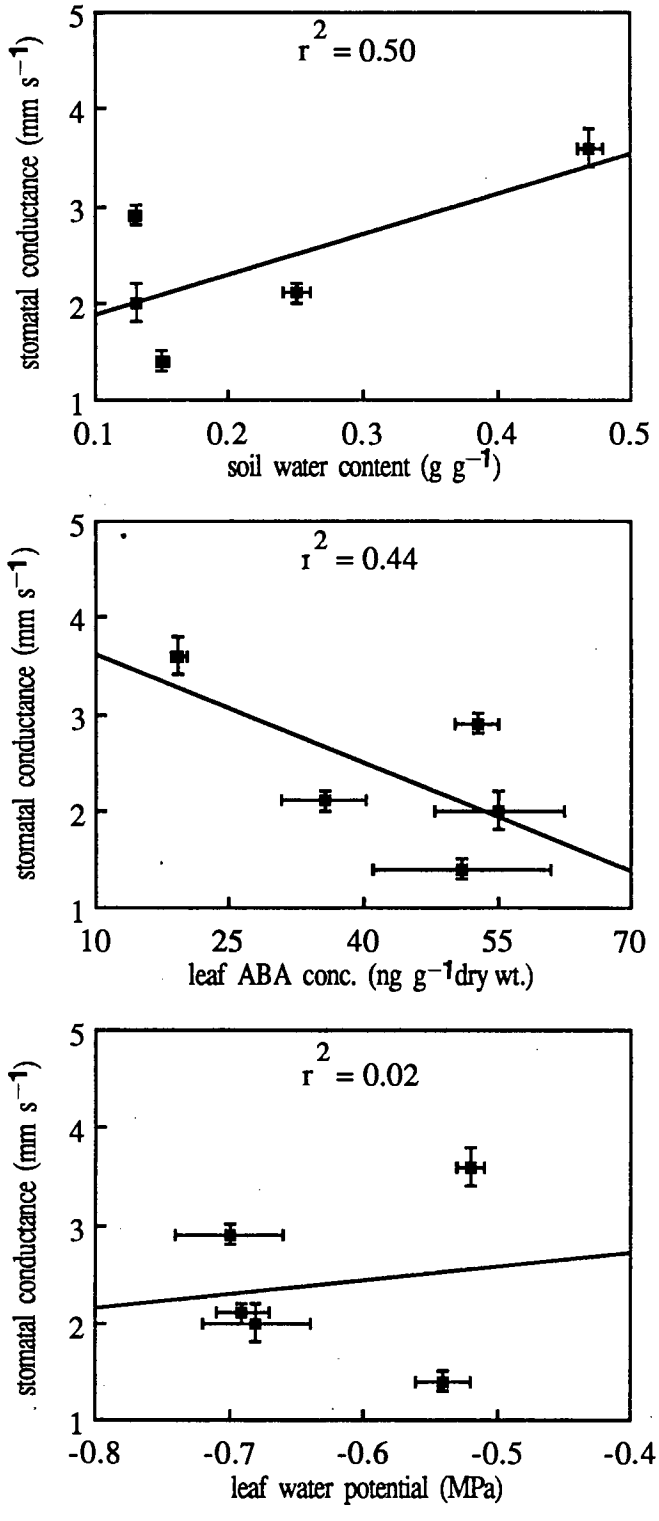

Figure 4.8: Linear relationships between abaxial stomatal conductance (n = 8) and soil water content (n = 4), leaf ABA concentration (n = 4) and leaf water potential (n = 6) in poplar plants. Points are means ± one standard error of the mean. Date were collected over a time course of soil drying, sampling the plants at 5-7 hours into the photoperiod of each day.

4.3.5 Stem and leaf growth and specific leaf area (SLA)

Compared to the control plants, stem elongation and leaf expansion of the treated plants decreased significantly with progressive soil drying. A significant decrease of stem elongation and leaf expansion was recorded in bean from day 6 and 4 of drying period, respectively (Fig. 4.9A and B) and in poplar from day 8 (Fig. 4.10A and B). In both bean and poplar plants, SLA was unaffected by soil drying and exceeded SLA of the control plants (Fig. 4.9C and 4.10C, respectively). Probably assimilate allocation was unaffected because of the continuous shoot water supply from the lower moist soil profile. Within two days of complete recovery of stomatal conductance in the treated poplar plants, the expansion of leaf area increased until it exceeded the values of the control plants (Fig. 4.11C), although no water was added from the top. At that time roots occupied significant volume of the lower moist soil column (Fig. 4.1 right).

4.3.6 Abscisic acid (ABA) concentration of leaves and roots

The ABA concentration of leaves and roots of the control plants of both species remained unchanged throughout the drying period in contrast to that of the treated plants where it increased significantly with time (Fig. 4.6). The ABA content of both leaves and roots was relatively larger in the bean plants than in the poplar plants during soil drying, particularly at the onset of drying, suggesting that the beans were more sensitive to soil water.

4.4 Discussion

Bean and poplar plants were grown separately in plastic pots containing soil columns, divided into an upper and a lower soil profile separated by a humid gap or gravel. With this design the root system of each plant was separated horizontally into the two different soil profiles in order to investigate the responses of growth and stomatal conductance during continuous drying of the upper soil profile, while some roots were able to explore the lower moist soil profile.

With progressive drying of the upper soil profile stomatal conductance declined gradually in both the bean and poplar plants whilst the bulk leaf water potential varied irregularly, suggesting control of the stomata independently of leaf water potential.

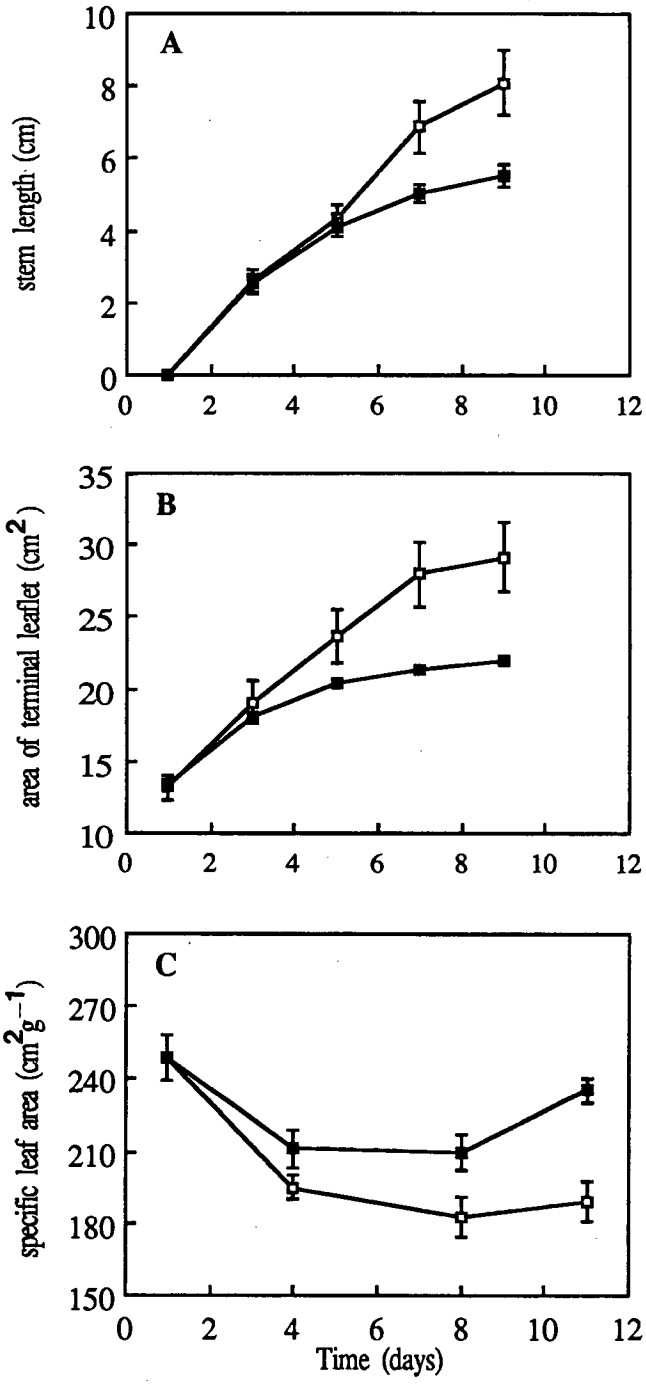

Figure 4.9: Increase in stem length (A) and area of terminal leaflet (B) and change in specific leaf area (C) of bean plants over the period of soil drying. □ well watered and ■ unwatered. Points are means of six measurements ± one standard error of the mean.

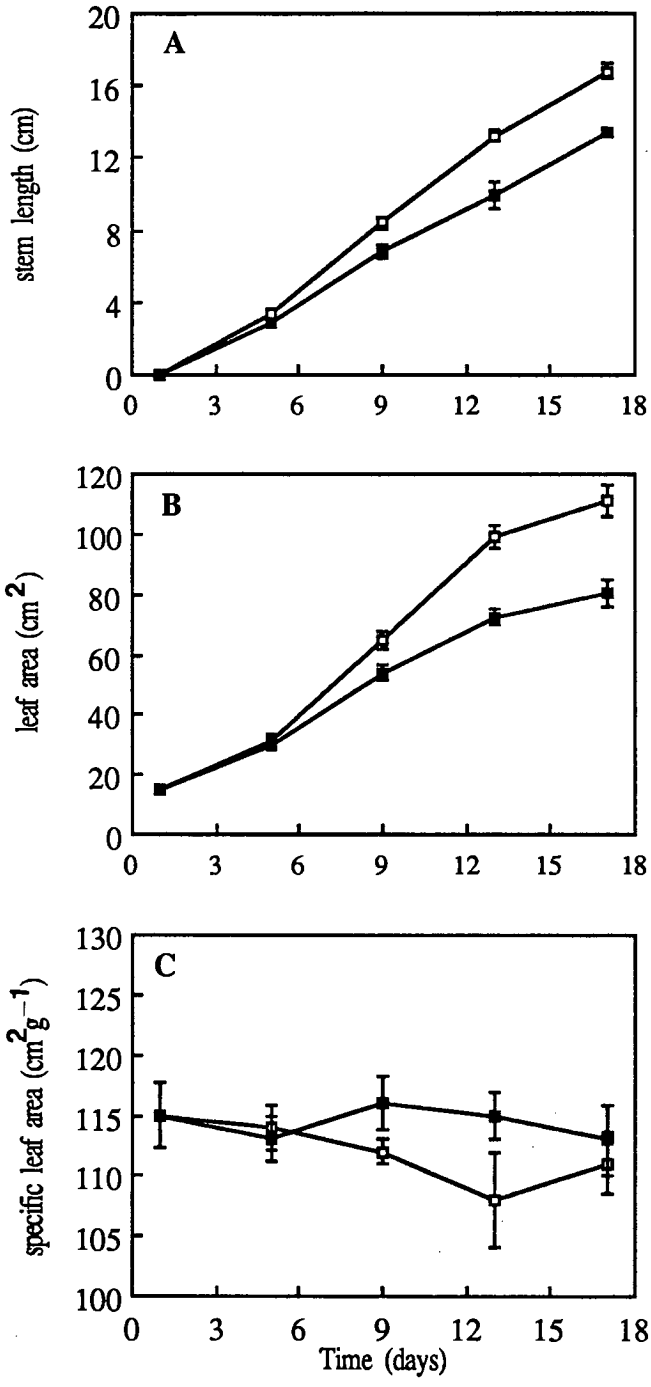

Figure 4.10: Increase in stem length (A) and leaf area (B) and change in specific leaf area (C) of poplar plants over the period of soil drying. □ well watered and ■ unwatered. Points are means of six measurements ± one standard error of the mean.

Much stronger relationships between stomatal conductance and soil water content than with leaf water potential (Fig. 4.7 and 4.8) supports this argument. The closure of stomata during soil drying in the absence of any detectable change of leaf water potential has been detected in several field and laboratory experiments (e.g. Bates and Hall, 1981; Blackman and Davies, 1985; Gollan *et al.*, 1986). Kramer (1988) has claimed that leaf water deficit is a common response to soil drying in the field and that such a change could provide a regulating influence on stomata. However, in an experiment on *Phaseolus vulgaris* L. (cv. Cacahuate-72 and Michoacan-12 A3) growing in a large volume of soil, stomatal closure was detected before any change in leaf water deficit (Trejo and Davies, 1991). The complete recovery of stomatal conductance of poplar treatment plants to values in excess of the control plants in the presence of significantly lower bulk leaf water potentials (Fig. 4.11A & B) supports the regulation of leaf water status by stomatal conductance rather than the reverse (Jones, 1985). However, just after the onset of soil drying (day 4 in bean and day 5 in poplar), bulk leaf water potential declined significantly although there was no substantial change of turgor potential in the poplar, in contrast to the bean (Fig. 4.4 & 4.5). Possibly the amount of roots in the moist soil profile was not adequate to maintain optimum shoot water supply, particularly in the bean. The reduction of water flow from the roots in the drying soil to the shoot, as the soil dried may also be the cause (Zhang and Davies, 1989b).

Leaf abscisic acid (ABA) concentration increased in treated plants of bean and poplar at the same time as stomatal conductance declined, suggesting control of the stomata by ABA, in agreement with the results of other studies (e.g. Zhang *et al.*, 1987; Zhang and Davies, 1989a, b). Possibly, ABA produced in dehydrating roots and transported by the xylem stream to the stomata may have led to stomatal closure (Davies and Zhang,1991). The build-up of ABA in roots in drying soil increased significantly relative to ABA in roots of control plants and it is likely that such ABA could move to the leaves in the transpiration stream (Zhang and Davies, 1987). By contrast, Jackson *et al.* (1988) disagreed with that view, when they argued that mature leaves rather than roots are the source of increased ABA content in young leaves and that this ABA was translocated to young leaves via the phloem. The current view is that generally ABA of unstressed leaves is trapped in the chloroplast but when water stress occurs, leaf water deficit first develops in older leaves which inject ABA into the xylem stream (Zhang and Davies, 1989b). In the present study the presence of lower bulk leaf water potentials just after the onset of soil drying leads to the reasonable possibility that the

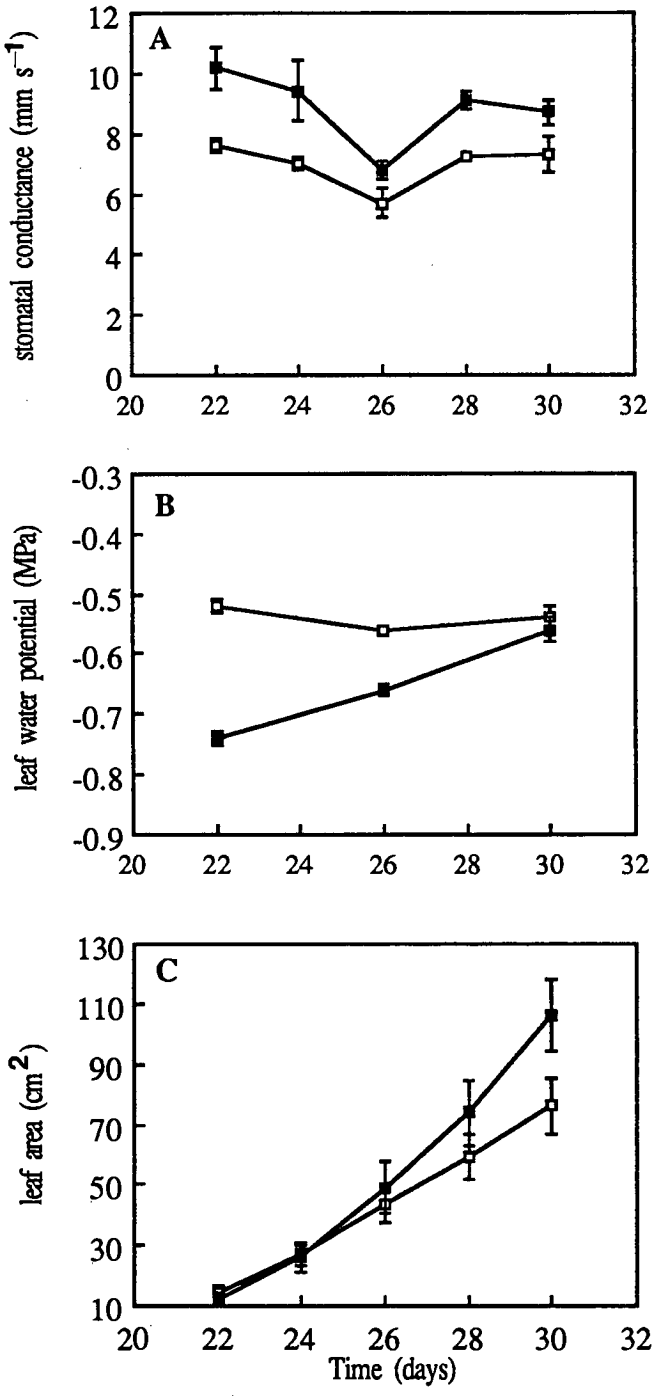

Figure 4.11: Change in abaxial stomatal conductance (A), leaf water potential (B) and leaf area (C) of poplar during the recovery period. □ well watered and ■ unwatered. Points are means of four measurements ± one standard error of the mean.

shoot contributes to the increase in leaf ABA. However, the source of leaf ABA is yet to be identified and needs to be investigated further in bean and poplar.

In both species the leaf ABA concentration gradually increased along with the gradual decline of soil water content, although the linear relationship between stomatal conductance and leaf ABA was not strong (Fig. 4.7 and 4.8). Variation in stomatal response to changed ABA concentration during soil drying as a result of environmental fluctuations in the greenhouse could explain this discrepancy. A similar weak relationship between leaf ABA content and stomatal conductance has also been observed in *Xanthium strumanium* (Cornish and Zeevaart, 1985).

As soil dries, tension is transmitted in the xylem water column of the shoot (Boyer, 1985) and may result in the interruption of the water potential gradient between the xylem and the growing cells (Boyer and Nonami, 1990). Therefore, the supply of water to the growing cells is reduced, turgor pressure declines and growth may be limited (Turner *et al.*, 1985). In these experiments, leaf expansion and stem elongation were affected concurrently with the decline of turgor potential in bean, although in poplar turgor was maintained at low water potential. Westagate and Boyer (1985) reported the reduction of leaf expansion in maize at low water potential despite turgor maintenance. The inhibition of shoot growth, which has also been observed in wheat (Passioura, 1988), maize (Saab and Sharp, 1989) and apple clones (Gowing *et al.* 1990) without change of shoot water status, may be the result of growth inhibition by ABA. The increased concentration of leaf ABA during soil drying found in the present study supports this idea. Zhang and Davies (1990b) also established a good correlation between shoot growth and xylem ABA concentration under soil drying. The regulation of shoot growth by endogenous ABA, may be attributable to the reduction of cell wall extensibility (Van-Volkenburgh and Davies, 1983; Kutschera and Schopfer, 1986) as reported in unwatered soybean seedlings (Bensen *et al.*, 1988).

The results of this experiment suggest that the growth of leaves and stomatal conductance in both bean and poplar plants is most likely influenced in a direct way by soil water status rather than by shoot water status. The simultaneous presence of lower bulk leaf water potential and higher root and leaf ABA contents over the period of soil drying indicates the possible coordinated influence of root signals and signals from water deficits in the leaves. These results provide support for further investigations to separate the origin of signals, whether from roots or shoot, during soil drying.

CHAPTER 5

Does ABA from Roots in Drying Soil Reduce Stomatal Conductance and Leaf Expansion in Bean and Poplar?

5.1 Introduction

Soil drying induces stomatal closure and inhibition of shoot growth, as observed in both bean and poplar plants (Chapter 3 and 4). In the conventional view, reduction in uptake of water from drying soil induces a reduction in leaf water potential which in turn influences the stomata and growth. However, there were no consistent changes in leaf water status during soil drying in the present work (Fig. 4.4 and 4.5). On the other hand, the relationship between stomatal conductance and soil water content was strong (Fig. 4.7 and 4.8), suggesting a possible indication of a non-hydraulic influence of soil drying on shoot functioning. Bates and Hall (1981) speculated that this was a chemical signal.

The simultaneous increase in ABA concentration in both roots and leaves during soil drying (Fig. 4.6) helps to explain the above results. The increasing concentration of ABA in roots in drying soil (Cornish and Zeevaart, 1985) may act as a signal to the shoots via the transpiration stream. However, ABA can also be released from leaves if turgor potential falls (Pierce and Raschke, 1980). This ABA could be redistributed with subsequent increase in leaf apoplastic ABA which results in stomatal closure (Zeevaart and Creelman, 1988). During soil drying the decline of leaf water potential in older leaves can also trigger ABA synthesis with consequent increase in ABA in the xylem water (Zhang and Davies, 1989b). Conversely leaf-synthesized ABA could be translocated to the roots in the phloem stream (Wolf *et al.*, 1990) and then transferred to the transpiration stream. Because of the initial decline of leaf water potential at the onset of soil drying, the source of increased ABA in leaves of the bean and the poplar, reported in earlier experiments (Chapter 4) remains uncertain.

The observation that stomatal closure can occur in plants with root systems in drying soil but without any significant leaf water deficit (Blackman and Davies, 1985; Gollan *et al*, 1986) suggests that the signal originates from the root environment. Blackman

and Davies (1985) in their experiment on maize plants did not find any increase of ABA concentration in leaves when parts of the root systems were in drying soil. They suggested that stomatal closure resulted from a decline in cytokinin transport from roots in drying soil. Conversely, in an experiment on *Commelina communis* with part of the root system in drying soil, Zhang *et al.* (1987) reported stomatal closure because of increased ABA concentration in the leaf epidermis. They suggested that a good correlation existed between leaf epidermal ABA and root ABA in drying soil because of a possible link between roots and leaf epidermis via the transpiration stream. In a separate experiment with *C. communis* seedlings, (Zhang and Davies, 1987) observed an increased concentration of ABA in leaf mesophyll and leaf epidermis when the root system was loaded with ABA solution. Thus the accumulation of ABA in leaves might account for stomatal regulation. However, Saab and Sharp (1989) did not find any reduction of stomatal conductance in maize plants with part of the root system in drying soil.

Like stomatal conductance, the inhibition of leaf growth in response to signals from the roots has also been reported in wheat (Passioura, 1988), maize (Saab and Sharp, 1989, Zhang and Davies, 1990a) and young apple clones (Gowing *et al.*, 1990). The decline in leaf growth of maize and sunflower, grown in large drying soil volumes showed a significant correlation with root-sourced ABA concentration in the xylem sap (Zhang and Davies, 1990b).

In both annuals (e.g. Blackman and Davies, 1985; Zhang *et al.*, 1987; Saab and Sharp, 1989) and young perennials (e.g. Gowing *et al.*, 1990) a sufficient shoot water supply can apparently be maintained with only half the root system in wet soil. With this in mind, experiments on bean and young poplar were conducted separately with vertically split root systems in separate pots, with one pot containing wet soil and the other drying soil. Stomatal conductance, leaf expansion, leaf water relations and root and leaf ABA concentrations were monitored with a view to detecting a possible increase in ABA concentrations in leaves in response to this partial soil drying, and whether the ABA originates in the roots or in the leaves.

5.2 Materials and Methods

5.2.1 Plant material and design of the experiment

Seedlings of bean and rooted cuttings of poplar were raised using the methods described in Section 4.2.1. The root system of bean seedlings (at two leaf stage) and rooted poplar cuttings (six weeks old) were divided, approximately equally by splitting the basal end of the stem vertically, using a sharp blade. In the case of bean, the main root attached to the base of the stem was also split. Each half of the root system of each plant was repotted into a separate pot containing compost (50% loam, 25% sand and 25% peat). The inside diameter of the pots was *ca* 84 mm and 150 mm (slightly less towards the bottom) with a height of *ca* 135 mm, for the growing bean and poplar plants, respectively. The stocks of each species with their split root system were kept in a small greenhouse (minimum day/night temperature 20/16 °C) with regular watering until they were considered to be established.

Established bean seedlings (at the stage of half expanded first trifoliate leaf) were transferred to a growth cabinet (Model 2340 G3, Fisons Scientific Apparatus, Loughborough, England) with a 12 hour photoperiod of 740 μmol m^{-2} s^{-1} photosynthetic photon flux density. The air temperature was 24 °C ± 2 during the day and 16 °C ± 2 at night, with relative humidity 70% ± 4. The treatment was started one week after transfer into these conditions. Established poplar cuttings were transferred to a large greenhouse (uncontrolled microclimate), where the treatment was started two weeks after transfer.

Both pots of each stock were watered every evening until the treatment started. From the day each experiment started (day 1), water was withheld from one of the two pots (with the other remaining well watered) of half of the plants (treatments) while both pots of the remaining plants were well watered every evening (controls) (Blackman and Davies, 1985). In both species, the growth of a well-developed root system in both pots was confirmed by initial harvesting of four, randomly selected plants. The layout of the plants in both experiments was a completely randomized design.

5.2.2 Measurement of soil water content

The whole soil volume was removed from the pot and samples were collected throughout, except on days 4 and 10 of the poplar experiment, when a metallic corer

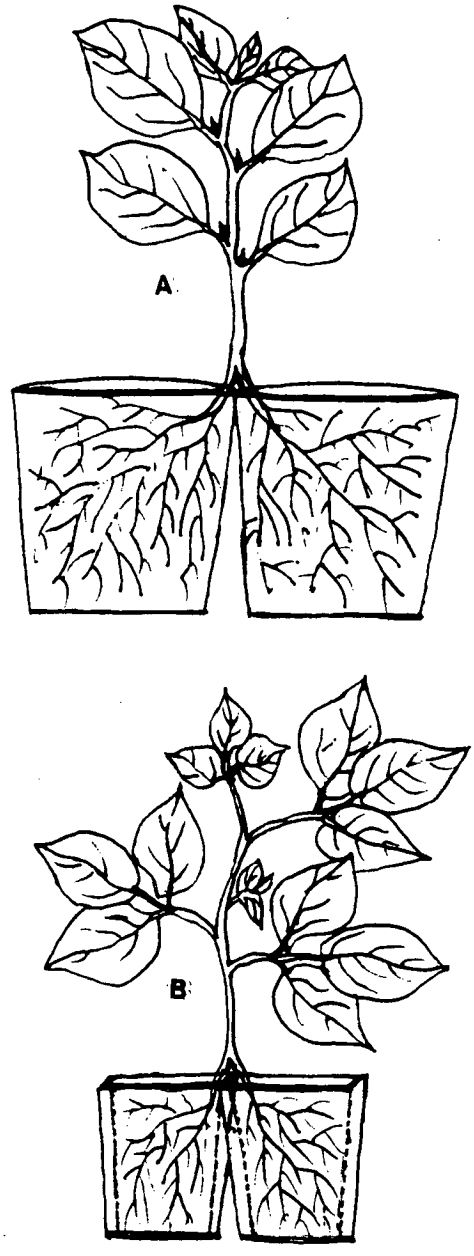

Figure 5.1: Diagramatic sketch of a poplar (A) and bean (B) plant grown with vertically split root systems, in two separate pots.

(15 mm diameter) was used. Soil samples were collected from the drying pot of the treatment and from any one pot of control. Four replicate measurements were made from each group (control and treatment) on each occasion (days 1, 4 and 8 in the case of bean and days 1, 4, 7, 10 and 14 in the case of poplar). Fresh and oven dry (at 90 °C for 48 hours) weight of the samples were recorded and the water content of soil was calculated gravimetrically.

5.2.3 Stomatal conductance

Measurements of abaxial stomatal conductance were made using a transient diffusion porometer (Delta-T Devices Ltd., Mark II, Cambridge, UK). The measurements were made as mentioned in Section 4.2.4. Eight plants of control and treatment of both bean and poplar were measured after five hours of photoperiod on days 1, 3, 4, 6 and 8 and on days 1, 4, 7, 10 and 14 of the drying cycles, respectively.

5.2.4 Leaf water relations

Measurements of leaf water relations were made on the day of measurement of stomatal conductance (except on day 3 of the bean experimental period) using the methods given in Section 4.2.5. In poplar the 5th or 6th leaf from the stem apex and in bean the terminal leaflet of the 1st or 2nd trifoliate leaf (depending on the day of measurement) was measured. Measurements were made on five bean and six poplar leaves of the control and treatment of each species, using one leaf from each plant.

5.2.5 Leaf expansion rate

Growing leaves and terminal leaflets of poplar and bean, respectively, were selected for the measurement. The length and width of the leaves were measured and later converted into areas (see Section 3.2.3). Measurements were made on six plants of control and treatment of bean and poplar on days 1, 3, 5, 7 and 8 and on days 1, 4, 7, 10 and 14 of the drying cycles, respectively. The same leaves/leaflets were measured throughout the period. The mean initial leaf size for control and treated plants was 24.40 ± 2.79 cm^2 and 23.95 ± 3.33 cm^2, respectively, in the case of bean and in the case of poplar 25.00 ± 2.74 cm^2 and 27.37 ± 2.98 cm^2. Daily leaf expansion rate was derived using $(A_2-A_1)/t$ (where A_1 and A_2 are initial and subsequent measurement of leaf area, respectively, and t is the interval of time) on each occasion of measurement. The expansion rate of each leaf of the treated plants was expressed

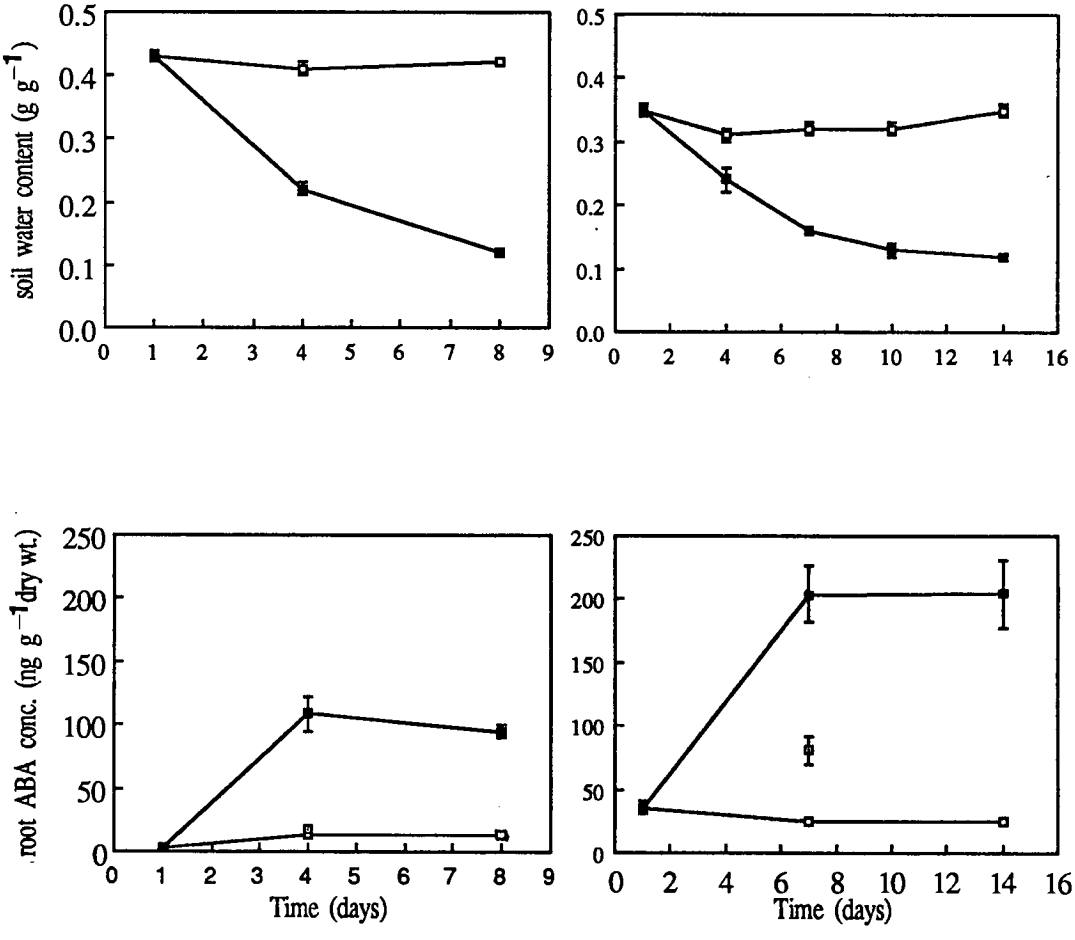

Figure 5.2: Decrease in soil water content and increase in root ABA concentration in drying pots of treatment (■) relative to control (□) plants over the experimental period for bean (left) and poplar (right). (⊡) root ABA in wet pots of treated plants. Points are means of four measurements ± one standard error of the mean.

as a percentage of the mean expansion rate of control plants.

5.2.6 Measurements of leaf and root abscisic acid (ABA)

The ABA concentration in the roots and leaves of both species were determined using the methods given in Section 4.2.9. Measurements of leaf ABA were made on the day of measurement of leaf water relations for both species. Root ABA concentration of bean and poplar was measured on days 1, 4 and 8 and on days 1,7 and 14 of the drying cycles, respectively. Root ABA in the wet pot of the treated plants was also measured on day 4 and 7 of the drying cycles for bean and poplar, respectively. Measurements were made on four replicates of control and treatment of each species.

5.2.7 Data analysis

Means and standard errors of the mean of each variable on each occasion were calculated and are presented in the form of graphs. Where the error bar of control and treatment means overlapped or were very close to each other, the paired data were tested by Students t-test, using the STATVIEW package.

5.3 Results

5.3.1 Soil water content and root ABA concentration

Withholding of water from one of the paired pots induced gradual decline of soil water content and a consequent rise in root ABA concentration (Fig. 5.2). Both these effects were significant relative to the well-watered controls. Surprisingly, the root ABA concentration in the wet pots of treated plants was observed to be higher relative to the control in both bean and poplar, on day 4 and day 7 of the drying cycles, respectively (Fig. 5.2), but the difference was only significant ($P < 0.05$) in the case of poplar. The concentrations of root ABA on days 4 and 8 in the case of bean and on days 7 and 14 in the case of poplar were similar.

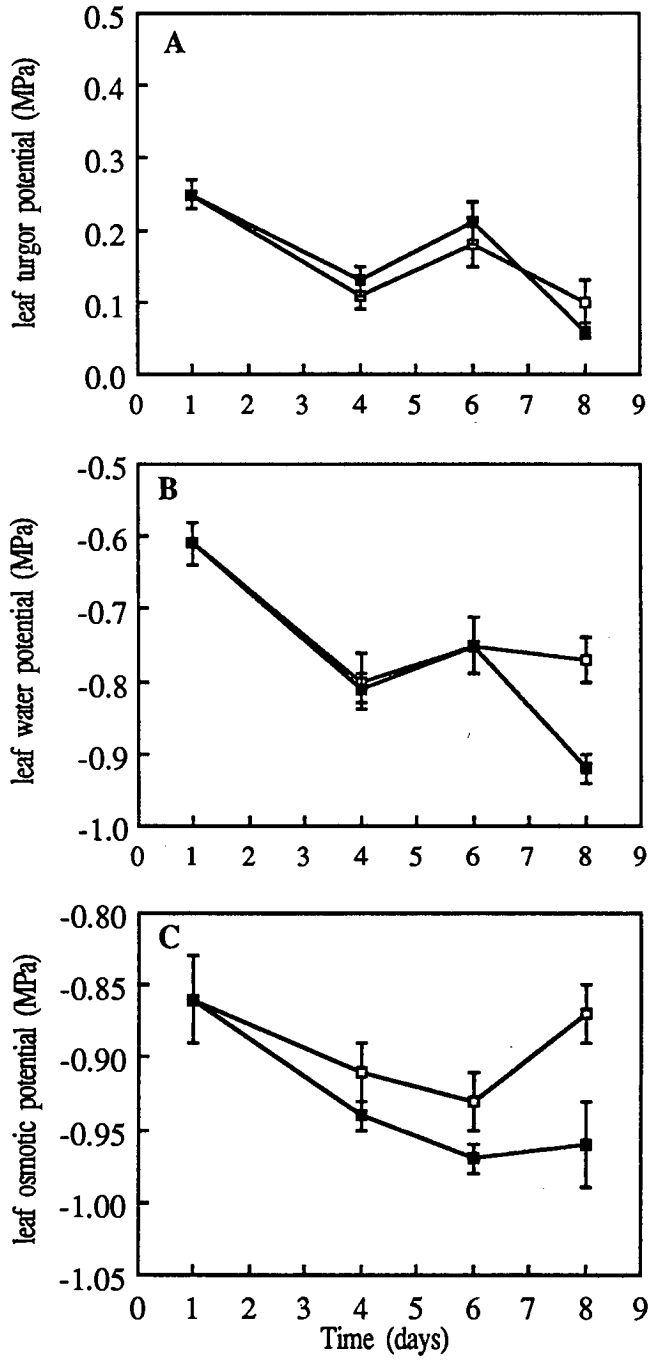

Figure 5.3: Change in turgor potential (A), water potential (B) and osmotic potential (C) of fully expanded bean leaves grown with vertically split root systems in two pots as a function of time. Water withheld from one half of the root systems from day 1, i.e. treated ■; water applied to both halves of the root systems, i.e. control □. Points are means of five measurements ± one standard error of the mean.

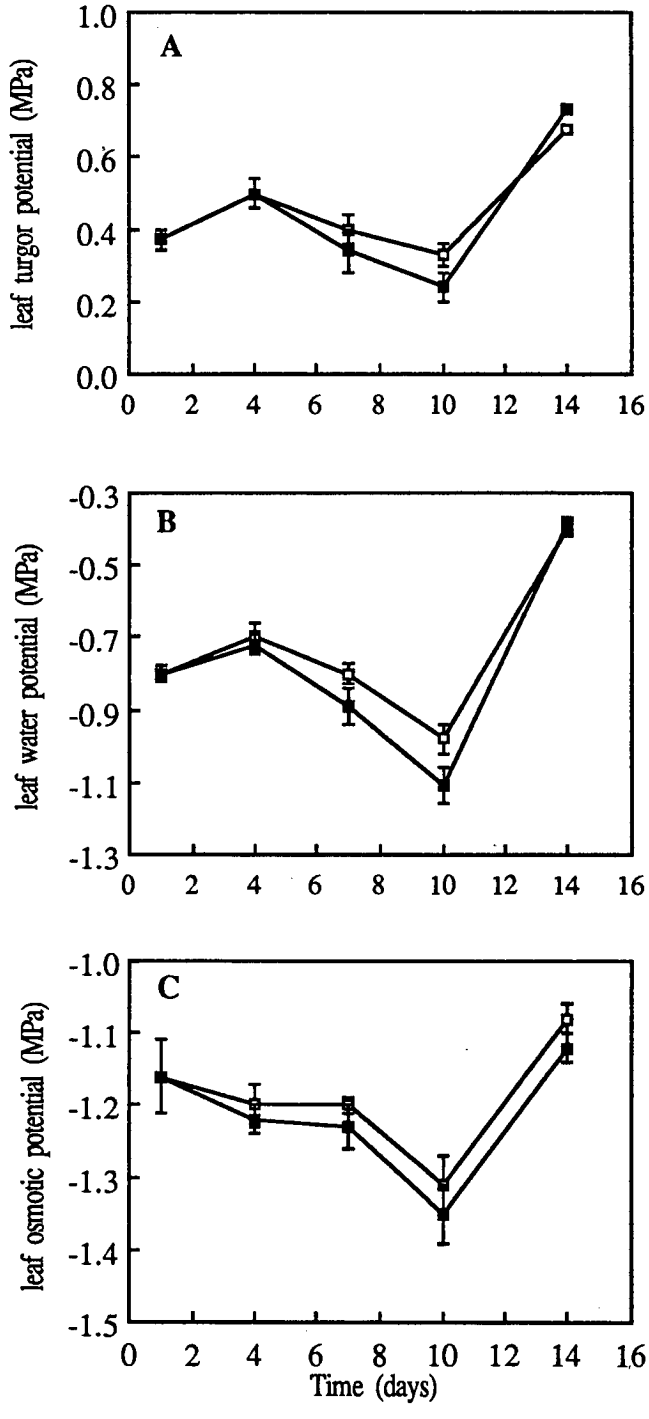

Figure 5.4: Change in turgor potential (A), water potential (B) and osmotic potential (C) of fully expanded poplar leaves grown with vertically split root systems in two pots as a function of time. Water withheld from one half of the root systems from day 1, i.e. treated ■; water applied to both halves of the root systems, i.e. control □. Points are means of six measurements ± one standard error of the mean.

5.3.2 Leaf water relations

Treated plants from which water was partially withheld, did not show significant differences in bulk leaf water potential and turgor potential from the control plants, in both bean and poplar during the first few days (until day 6 and 7, respectively) of the drying cycles (Fig. 5.3 and 5.4). Presumably the root systems in the wet pot of the treated plants were sufficient to maintain shoot water supply as suggested in Section 5.1. However, substantial reductions in bulk leaf water potentials and turgor potential were observed in both bean and poplar on day 8 and day 10 of the drying cycles, respectively (Fig. 5.3 and 5.4).

5.3.3 Stomatal conductance and leaf ABA concentration

Treated plants, with part of their root systems in continuously drying soil, showed gradual decline of abaxial stomatal conductance in association with the simultaneous increase of leaf ABA concentrations (Fig. 5.5 and 5.6) without any initial change in bulk leaf water potential and turgor potential (Fig. 5.3 and 5.4). This suggests that root synthesized ABA was controlling stomata. Significant recovery of stomatal conductance was observed in both bean and poplar (95% and 85% of control plants, respectively) on day 8 and day 14 of the drying cycles, respectively (Fig. 5.5 and 5.6). At that time leaf ABA concentration in treated plants of bean and poplar also decreased closed to the level in the control plants (102% and 118% of control plants, respectively), again suggesting a significant effect of leaf ABA on stomatal conductance.

Plotting relative stomatal conductance of treated plants (expressed as % of mean value of control plants) against relative increase in leaf ABA concentrations (mean value of treatment/mean value of control) also showed a linear relationship between these two variables (Fig. 5.7). This relationship was rather stronger in the bean plants (r^2 = 0.96) than in the poplar plants (r^2 = 0.69), perhaps because of higher sensitivity of bean stomata to leaf ABA. However, in poplar, there was a significant initial (day 4) decline in stomatal conductance before the leaf ABA concentration increased significantly (Fig. 5.6).

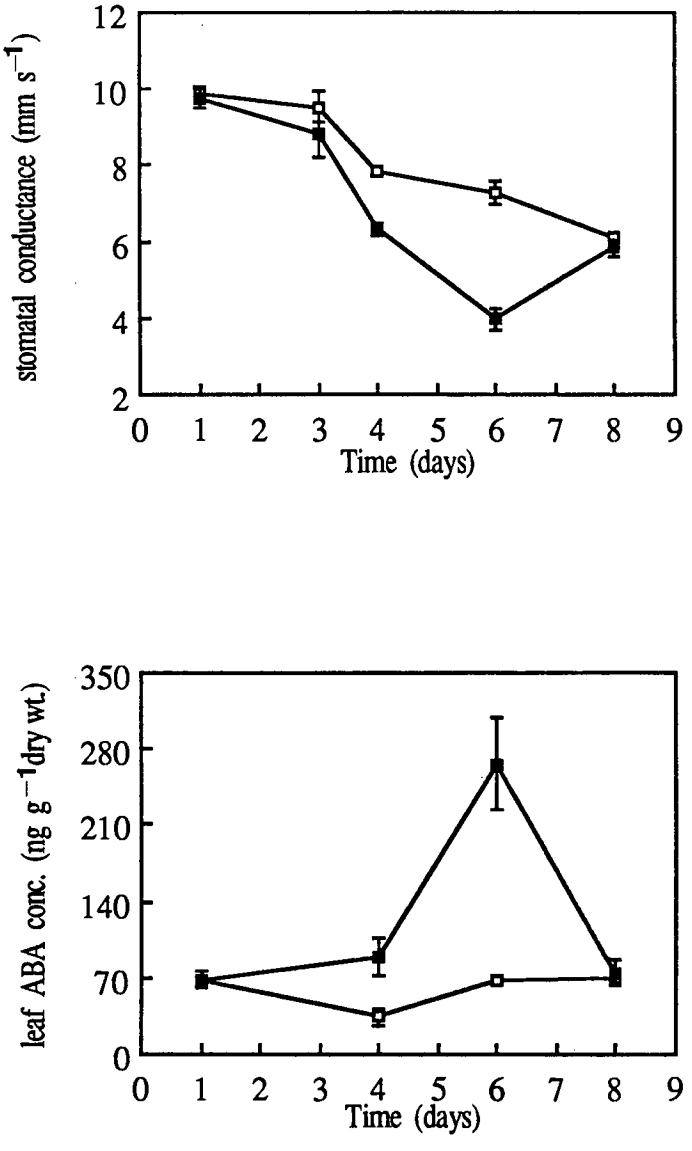

Figure 5.5: Change in abaxial stomatal conductance and ABA concentration in leaves of bean grown with vertically split root systems in two pots as a function of time. Water withheld from one half of the root systems from day 1, i.e. treated ■; water applied to both halves of the root systems, i.e. control □. Points are means of eight and four measurements for stomatal conductance and leaf ABA, respectively ± one standard error of the mean.

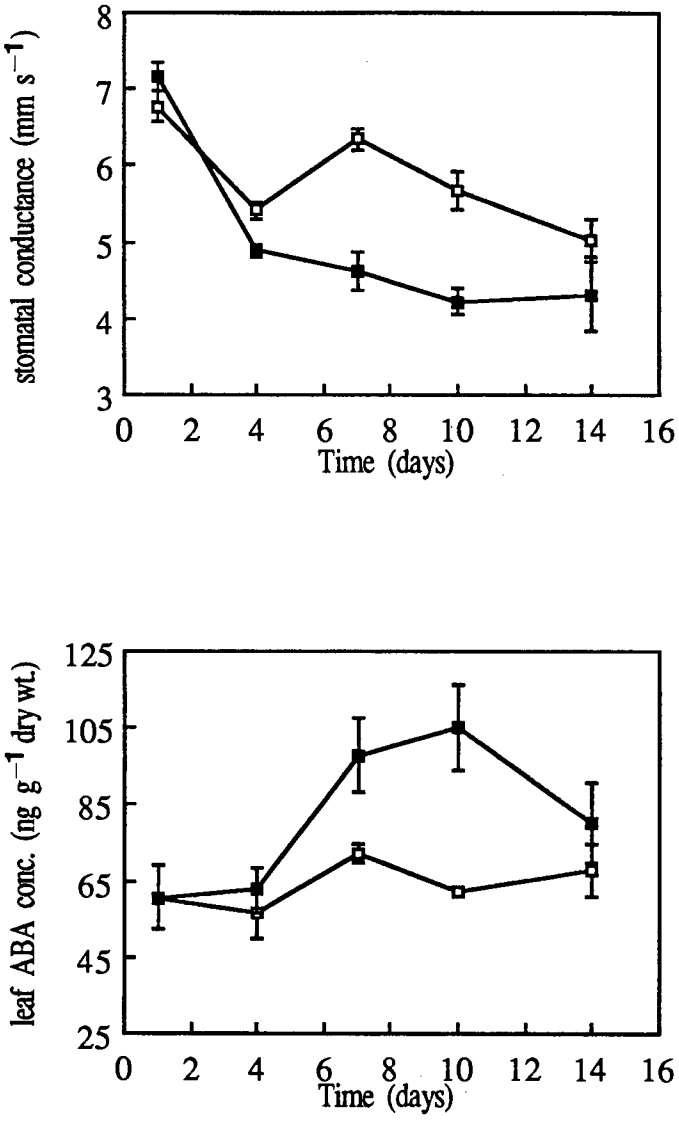

Figure 5.6: Change in abaxial stomatal conductance and ABA concentration in leaves of poplar grown with vertically split root systems in two pots as a function of time. Water withheld from one half of the root systems from day 1, i.e. treated ■; water applied to both halves of the root systems, i.e. control □. Points are means of eight and four measurements for stomatal conductance and leaf ABA, respectively ± one standard error of the mean.

5.3.4 Leaf growth

The leaf expansion rate of treated plants (expressed as percentage of mean value of control plants) decreased progressively in association with the increase in leaf ABA and the reduction of stomatal conductance during the period of soil drying (Fig. 5.8). In treated plants of both bean and poplar, the rate of leaf expansion was found to be approximately 22% lower than for the control plants after day 7 and day 10 of the drying cycles, respectively. The reduction was small (approximately 10-12%) and not significant before then (Fig. 5.8).

5.4 Discussion

As predicted from previous experiments (Chapter 4), shoot water supply is of prime importance in detecting the influence of soil drying on shoot physiology in the absence of any change in shoot water status. Both bean and poplar plants with approximately half of the root systems in drying soil did not show any change in leaf turgor potential and bulk leaf water potential, relative to control plants, at least for the first few days (Fig. 5.3 and 5.4), in agreement with the results of other split root studies (Blackman and Davies,1985; Zhang *et al.*, 1987; Saab and Sharp, 1989; Gowing *et al.*, 1990). The possibility of maintaining optimum shoot water supply by a proportion of the roots must depend on the amount of roots in the moist soil. The closure of stomata in both the species in the presence of such unchanged leaf water status agrees with the hypothesis that non-hydraulic signals originate from roots in drying soil (Gollan *et al.*, 1986).

Generally, root systems surrounded by drying soil may lose their capacity to take up soil water because of resistance to soil water movement at the interface between roots and soil. As a result, root tips lose their turgor potential where, it is proposed, the production of ABA is eventually triggered (Zhang and Davies, 1989a). The significant increase of ABA in roots in dry soil relative to roots in wet soil in both species reported here (Fig. 5.2) can be a similar consequence of soil drying. It has been proposed that this ABA is subsequently transported to the leaves in the transpiration stream resulting in increased accumulation of ABA in the leaves (Zhang and Davies, 1987; Neales *et al.*, 1989). In the bean and poplar plants with part of the root system in drying soil, the gradual decline in stomatal conductance with simultaneous increase in bulk leaf ABA clearly indicates an inhibitory effect of bulk

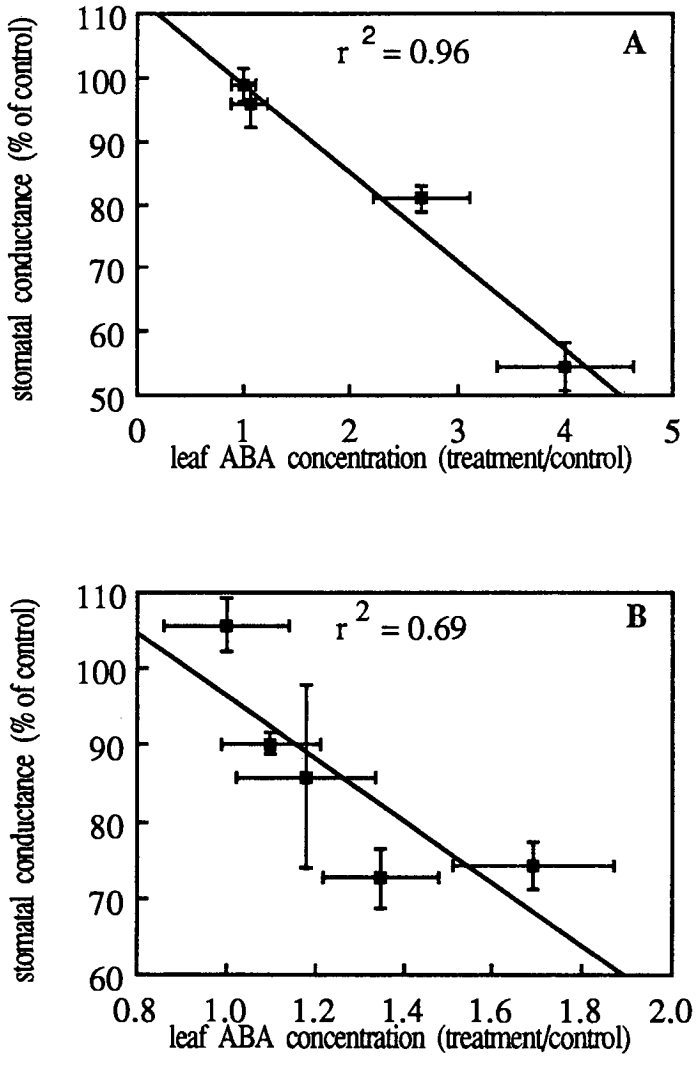

Figure 5.7: Relative abaxial stomatal conductance as a function of relative increase of bulk ABA concentration in leaves of bean (A) and poplar (B). Points are results from Figure 5.5 and 5.6. (Stomatal conductance is expressed as a percentage of the mean value of the control plants; ABA concentration is divided by the mean value of the control plants) with ± one standard error of the mean. Regression line and value of coefficient of determination (r^2) are shown.

leaf ABA on stomata, possibly by regulating stomatal guard cells (Davies and Zhang, 1991). Similar effects of root-sourced ABA on stomatal closure have also been reported in several other studies of partial soil drying, using methods where plants were grown in large soil volumes and with split root systems in separate pots (e.g. Zhang *et al.* 1987; Zhang and Davies, 1989a & b, 1990). However, Beardsell and Cohen (1975) reported the closure of stomata in maize and sorghum under soil water stress before the increase of bulk leaf ABA.

In an experiment on *C. communis* with part of the root system in drying soil, increased ABA in the leaf epidermis reduced stomatal conductance (Zhang *et al.*, 1987). They suggested that incoming ABA from roots in drying soil is redistributed in the leaves and that enhanced epidermal ABA coincides with the increased ABA contents of the roots. Since ABA from water-stressed roots is transported to the leaves continuously in the transpiration stream, ABA is likely to accumulate gradually in the apoplast of transpiring leaves and could influence stomatal guard cells in due course. Although apoplastic ABA must increase before cellular ABA during water stress, with the increase in bulk leaf ABA larger quantities of ABA become readily available to the guard cells (Cornish and Zeebaart, 1985). Thus, it is resonable to suggest the influence of increased bulk leaf ABA on stomatal closure. The apparent recovery of stomatal conductance at the end of the drying cycle, in association with a significant decline in bulk leaf ABA in the treated plants of both bean (Fig. 5.5) and poplar (Fig. 5.6), is also a strong argument in favour of a significant role for bulk leaf ABA on stomata.

In contrast to the poplar, the concentrations of ABA in the leaves of bean were rather higher than in the roots, possibly because of more rapid transport of ABA from roots to leaves and accumulation in the leaves. The higher stomatal conductance of bean relative to poplar (Fig. 5.5 and 5.6) may indicate more rapid transport of ABA because of a higher transpiration rate, although transpiration rate was not measured in these experiments. If transpiration rates were different, other possible differences between the two species in the transport of ABA between roots and shoot, as well as in redistribution from leaves to the roots, are possible. The presence of more ABA in roots in wet pots of treated plants, relative to control plants (significant in poplar) could result from the translocation of ABA from leaves to roots in the phloem stream (e.g. Zeevaart and Boyer, 1984; Wolf *et al.*, 1990), which may have been greater in poplar.

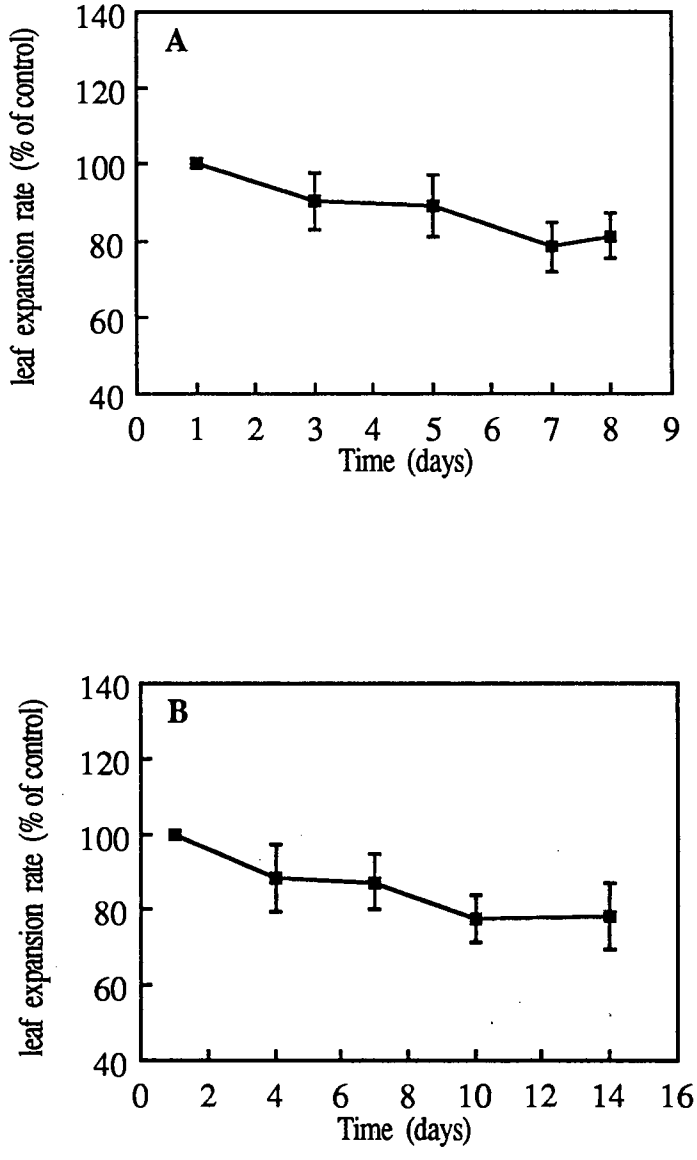

Figure 5.8: Daily leaf expansion rate of bean (A) and poplar (B) plants with water withheld from one half of the root systems, expressed on each occasion as a percentage of the mean leaf expansion rate of plants with both halves of the root systems well watered. Points are means of six measurements ± one standard error of the mean.

Recovery of stomatal conductance at the end of the drying cycle in both species suggests that ABA may not be being transported from the roots in the dry pots, because of the lack of available water in the excessively dry soil (soil water content 0.12 g g^{-1}). As a result, the ABA concentration previously accumulated in the leaves of the treated plants is not reinforced and is diluted with the continuous flux of water from the wet pots. Thus the stomata may also be sensing the relative water flux through the different parts of the root system, in addition to sensing the soil water status (Tardieu *et al.*, 1992a).

The initial (day 4) decline of stomatal conductance in poplar in the absence of any significant increase of bulk leaf ABA (Fig. 5.6) may be explained by extreme stomatal sensitivity to a small change in leaf ABA. Wartinger *et al.* (1990), in a field experiment on almond trees grown in a desert, observed stomatal sensitivity to a very small range of ABA concentration. Zhang and Davies (1989a &b) suggested higher sensitivity to soil drying of maize and sunflower to xylem sap ABA rather than to bulk leaf ABA. They did not find any increase in leaf ABA under soil drying within the first few days of the drying cycle, although xylem sap ABA significantly increased. So, it would be worth investigating the change in xylem sap ABA with soil drying in bean and poplar plants. However, the influence of an unidentified regulator of stomatal behaviour can not be ruled out (Trejo and Davies, 1991). The closure of stomata in the absence of any increase in ABA in the shoot has also been reported elsewhere (e.g. Blackman and Davies, 1985; Munns and Kings, 1988).

Cell enlargement and thus leaf expansion is driven by turgor and so depends on water balance. Soil drying induces a reduction in growth as a result of reduction in cell turgor potential, at least in some cases (Kramer, 1983), but reduction in leaf growth has also been reported in wheat despite turgor maintenance (Passioura, 1988). In the present study, in both bean and poplar plants with part of the root systems in drying soil, the leaf expansion rate gradually declined (Fig. 5.8) in association with the increase in bulk leaf ABA (Figs 5.5 and 5.6), in spite of only small changes in leaf turgor potential and bulk leaf water potential (Figs. 5.3 and 5.4). This also provides further support to the hypothesis of a non-hydraulic influence of roots in drying soil on the shoot and is in agreement with the results of other split root experiments on maize (Saab and Sharp, 1989) and young apple clones (Gowing *et al.*, 1990) where part of the root systems were also subjected to soil drying.

However, withholding water from part of the root systems could induce an immediate

hydraulic tension in the xylem sap resulting in a possible interruption in the water potential gradient between xylem and growing cells in expanding leaves (Boyer, 1985). As a result, water supply to expanding cells could eventually decline, thus reducing the driving force for expansion of the cells (Boyer and Nonami, 1990). But, because of the continuous water flux from roots in the wet pot, such a tension in the xylem seems unlikely. Here the gradual increase of bulk leaf ABA in the treated plants, despite turgor maintenance, suggests an inhibitory effect of ABA on leaf expansion. The apparent start of recovery of leaf expansion in the treated plants of bean (data not shown) from the day of recovery of stomatal conductance (when leaf ABA was similar (102%) to that of control plants) supports the argument. Since changes in cell wall properties under drought can control leaf growth (Rhizopoulou, 1990), the increased leaf ABA concentration could act on the wall of growing cells, resulting in a reduction in cell wall extensibility (Kutschera and Schopfer, 1986).

The results reported here suggest that a positive root-sourced signal, apparently bulk leaf ABA, is capable of inhibiting stomatal conductance and leaf expansion of bean and poplar plants. Therefore, these early responses of plants to partial soil drying could enable them to measure soil water status (Turner *et al.*, 1985) before any seriously damaging effects of water deficit. This mechanism may allow better utilization of soil water by plants in areas where water shortages occur (Jones, 1980; Cowan, 1982).

Correlation between ABA and conductance does not imply a cause-and-effect relationship. Ideally further studies in which ABA is controlled experimentally are required.

CHAPTER 6

Increase in Xylem Sap ABA in Response to Soil Drying Regulates Stomatal Conductance and Leaf Growth in Bean and Poplar

6.1 Introduction

Evidence of an inhibitory effect of soil drying on stomatal conductance and leaf expansion in the absence of any loss of leaf turgor or bulk leaf water potential has recently been established (Blackman and Davies, 1985; Gollan *et al.*, 1986, Passioura, 1988; Zhang and Davies, 1990a). A chemical signal in the transpiration stream which originates from roots in drying soil, was first hypothesized in field grown cow-pea (Bates and Hall, 1981) and later postulated in greenhouse-grown maize, based on the split root system technique (Blackman and Davies, 1985). Abscisic acid (ABA) plays a major role in chemical signalling from root to shoot (Zhang *et al.*, 1987). Synthesis of ABA is generally enhanced in dehydrating roots because of turgor loss of the root tip (Zhang and Davies, 1987; Neales *et al.*, 1989). In a gradual soil drying situation, shallow roots generally start to dehydrate quickly because of the high concentration of surface roots and excessive surface evaporation while roots in deeper moist soil maintain a sufficient shoot water supply. These dehydrating shallow roots may trigger production of ABA which is transported to the shoot in the transpiration stream with a resulting reduction of stomatal conductance and leaf growth, without any change in leaf water status (Zhang and Davies, 1990a).

The present study (Chapter 5) suggests an inhibitory influence of ABA from the roots on stomatal conductance and leaf growth of bean and poplar plants with part of their root system in drying soil. Zhang *et al.* (1987) in their experiment on *Commelina communis* with part of the root system in drying soil (using a split root system technique) suggested that there was a correlation between leaf epidermal ABA (not bulk leaf ABA) and increased ABA content of the roots in drying soil. In a separate experiment with *C. communis* with their root system loaded by external ABA, an increase in both leaf epidermal and mesophyll ABA was detected (Zhang and Davies, 1987). However, in our study on both bean and poplar (Chapter 5), the reduction of stomatal conductance was accompanied by a simultaneous increase in bulk leaf ABA concentration although the initial (day 4 of the drying cycle) significant decline in

stomatal conductance of poplar occurred before there was a significant increase in bulk leaf ABA (Fig. 5.5 and 5.6).

Since ABA from roots is transported to the leaves in the transpiration stream, the ABA concentration of xylem sap should be increased *before* the increase in bulk leaf ABA, as apparently was observed in maize and sunflower plants (Zhang and Davies, 1989a & b). From the results with maize they suggested that the increased concentration of xylem sap ABA was ultimately large enough to cause the substantial increase in bulk leaf ABA observed from day 9 of a 21-day drying cycle. But in a separate experiment on maize plants grown under almost similar conditions there was no significant difference in bulk leaf ABA concentration of well-watered and unwatered plants during the 20 days of a drying cycle (Zhang and Davies, 1990a). These discrepancies in the change of bulk leaf ABA under soil drying might be explained by the redistribution of ABA in and out of the leaf as a result of changes in pH gradient between cytoplasm, apoplast and phloem vessels (Hartung, 1976; Hartung *et al.*, 1990). Nevertheless, xylem sap ABA has a direct link via the leaf apoplast, to the stomatal guard cells and thus to the stomatal response to ABA from the roots. Therefore, the relationship between stomatal conductance and xylem sap ABA might be expected to be stronger than the relationship with bulk leaf ABA (Tardieu *et al.*, 1992b). However, because of stomatal sensitivity to both leaf apoplastic ABA and leaf epidermal ABA, bulk leaf ABA itself may appear to be involved in stomatal regulation.

Munns and King (1988) questioned the role of xylem sap ABA in controlling stomatal conductance. They detected a reduction in transpiration of detached wheat leaves fed on ABA-free xylem sap. The closure of stomata in the absence of xylem sap ABA has also been reported in *Phaseolus vulgaris* grown in large gradually-drying soil volumes (Trejo and Davies, 1991). On the other hand, in sunflower and maize plants reduction of leaf growth and stomatal conductance has been correlated with increased xylem sap ABA concentration when the shallow parts of the root system were dehydrating (Zhang and Davies, 1989a & b, 1990a & b). However, Tardieu *et al.* (1992a) explained the different situation in the field, where a significant increase in xylem sap ABA was correlated with a reduction of stomatal conductance, by cumulative drying of the whole soil profile rather than drying of the shallow soil layer. Thus, the process of soil drying may regulate the change in xylem sap ABA concentration and may be species specific. Consequently, in a progressively drying soil possible changes in xylem sap ABA concentration of bean and poplar plants need to be clarified, with a view to evaluating the role of xylem sap ABA in regulating stomatal conductance and

leaf growth.

The present experiment was conducted with bean and poplar plants separately, grown in progressively drying, large soil volumes (*ca* 4000 cm^3) under similar microclimatic conditions to address the question of whether the concentration of xylem sap ABA, rather than bulk leaf ABA, provides a better explanation of the reduction in stomatal conductance and leaf growth. Here possible differences between the two species in their response under similar growing conditions were investigated with a view to identifying the specific response characteristics of annuals and young trees.

6.2 Materials and methods

6.2.1 Plant materials and design of the experiment

Seedlings of bean and rooted cuttings of poplar were raised using the methods described in Section 4.2.1. The seedlings of bean (at two leaf stage) and the rooted cuttings of poplar (4 weeks old) were transplanted into separate pots of 16.8 cm inside diameter containing *ca* 4000 cm^3 of compost (50% loam soil, 25% sand and 25% peat). All the plants were watered regularly and raised in a small greenhouse with a minimum day/night temperature of 20/16 °C until they were established. The established seedlings of bean (at the stage of one nearly fully expanded trifoliate leaf) and cuttings of poplar (approximately uniform size with a flush of new growth) were transferred to a growth cabinet. The experiments with bean and poplar plants were conducted separately at different times inside the same growth cabinet with similar growth conditions for the same period (8 days) of a drying cycle. In each experiment, all the plants were watered every evening until the treatment started. From the day each experiment started (day 1) half of the plants were watered every evening (control) and the other half (the treatment) received no water. Both control and treatment plants were placed in a completely randomized design on the bench of the growth cabinet. In the case of the poplar the treatment was started when the cuttings had 4-6 fully expanded leaves and in the case of bean when the seedlings had the first fully expanded trifoliate leaf.

In both experiments, measurements were made of soil water content, abaxial stomatal conductance, leaf water relations, leaf and xylem sap ABA concentration and leaf expansion. On day 1 of the drying cycle, initial measurements of all variables were

made on four plants, and on days 3, 5 and 8 of the drying cycle measurements of all variables were made on four plants of both the control and treatment. On each occasion, measurements of all variables were made on the same plants. In the case of bean, total leaf area was also measured on each occasion (days 1,3,5 and 8).

6.2.2 Growth cabinet and growth conditions

In the growth cabinet the plants were illuminated with metal halide fluorescent lamps (Kolorarc 400 W MBIF/H, Thorn Lighting, London, UK) and with 60 W incandescent bulbs. On the bench the PPFD was 350 μmol m^{-2} s^{-1}. Other growth conditions were, 12 h photoperiod with day and night temperatures 24 and 16 ± 2 °C, respectively. The air flow of about 1 m s^{-1} was horizontal with relative humidity of 70 ± 5%.

6.2.3 Soil water content

The whole soil column was removed from the pot and samples were collected from upper and lower layers of the soil column separately. Fresh and oven dry (at 90 °C for 48 hours) weight of the samples were recorded and the water contents of soil in each layer were calculated gravimetrically: the overall water content of the whole soil column is presented.

6.2.4 Stomatal conductance

Measurements of stomatal conductance were made on the abaxial surface of leaves after 5 hours of photoperiod using a transient diffusion porometer (Delta-T Devices Ltd., Mark II, Cambridge, UK) as described in 4.2.4.

6.2.5 Leaf water relations

Measurements of leaf water relations were made using the methods described in Section 4.2.5.

6.2.6 Leaf expansion rate

Leaf area was measured and later daily leaf expansion rate was calculated using the methods described in Section 5.2.5. In both control and treated plants, the expansion

rate of each leaf was expressed as a percentage of the mean expansion rate of leaves on the control plants. The mean initial leaf area of four leaves for control and treatment plants was 22.91 ± 3.76 cm^2 and 22.21 ± 3.08 cm^2, respectively, in the case of bean and in the case of poplar 23.26 ± 5.36 cm^2 and 22.95 ± 5.00 cm^2.

6.2.7 Total leaf area

In the experiment with bean plants the total leaf area was measured destructively on each occasion (days 1, 3, 5 and 8) using a leaf area meter (LI 3100, LiCor, Inc. Lincoln, USA). The initial mean total leaf area of four randomly selected plants was 253 ± 10 cm^2 per plant.

6.2.8 Measurement of abscisic acid in leaves and xylem sap

Leaf samples were collected and frozen for ABA measurement using methods described in Section 4.2.9. The xylem sap was collected by pressurizing the stump of the whole root system using a pressure chamber (Zhang and Davies, 1989a). Plants were first cut at the apex to release the tension in the xylem and then cut again leaving a stump of about 10-15 cm length (depending on the length available) above the soil surface. The stump with the whole root system, including some attached soil particularly from the lower layer, was removed carefully with minimum damage to the root system and placed in a small pressure chamber with the cut end of the stump protruding. By applying a pressure of -0.5 to -0.8 MPa, *ca* 110-120 mm^3 of sap was collected from one plant in a Eppendorf vial within 6 to 8 minutes and frozen in liquid nitrogen. The samples of leaves and xylem sap were stored in a freezer (-80 °C) and later used in the determination of ABA concentration using the radioimmunoassy (RIA) protocol (Appendix II), described by Quarrie *et al.* (1988). The xylem sap was used directly in the assay. Leaf samples from which ABA was extracted overnight were oven dried (at 90 °C) later to get the dry weight of each sample. The results are expressed as ng of ABA per g of dry weight of sample for leaf ABA and µmol m^{-3} of xylem sap for xylem sap ABA.

6.2.9 Determination of relationship between various variables

For both species, changes in ABA concentration of xylem sap and leaves of treated plants were plotted against the resultant soil water content during the whole drying cycle (days 1, 3, 5 and 8). Similarly, stomatal conductance and leaf expansion rate (%

Table 6.1: Changes in SWC, XS ABA, L ABA, ASC, LWP, LTP, LOP and LER with time when bean plants were grown in large soil volumes (*ca* 4000 cm^3) after withholding water from the soil. Two tailed paired student t-test. Values are means of four observations ± one standard error of mean.

Variables	Time (days)									
	1	3			5			8		
		WC	WS	P<	WC	WS	P<	WC	WS	P<
SWC ($g\ g^{-1}$)	0.38 ±0.01	0.39 ±0.01	0.30 ±0.01	0.05	0.36 ±0.01	0.24 ±0.01	0.05	0.35 ±0.02	0.18 ±0.01	0.001
XS ABA (μ mol m^{-3})	82.83 ±3.50	69.81 ±5.67	158.13 ±21.78	0.05	86.61 ±6.16	202.79 ±21.96	0.05	91.79 ±8.44	263.04 ±17.40	0.01
L ABA (ng g^{-1} dw)	10.60 ±1.09	17.40 ±1.18	22.62 ±3.93	ns	24.01 ±2.34	64.95 ±8.70	0.05	22.62 ±2.38	66.09 ±9.06	0.05
ASC (mm s^{-1})	9.70 ±0.18	8.14 ±0.17	6.90 ±0.14	0.05	8.30 ±0.32	6.00 ±0.16	0.01	8..91 ±0.11	3.53 ±0.14	0.001
LWP (- MPa)	0.65 ±0.02	0.57 ±0.03	0.60 ±0.03	ns	0.62 ±0.02	0.63 ±0..01	ns	0.65 ±0.01	0.85 ±0.02	0.001
LTP (MPa)	0.30 ±0.04	0.34 ±0.03	0.33 ±0.01	ns	0.35 ±0.04	0.32 ±0.05	ns	0.34 ±0.01	0.25 ±0.02	0.05
LOP (-MPa)	0.94 ±0.02	0.91 ±0.02	0.93 ±0.03	ns	0.96 ±0.03	0.95 ±0.05	ns	0.99 ±0.02	1.10 ±0.03	ns
LER (% cont.m)	100.00 ±0.00	100.01 ±5.69	84.13 ±4.40	ns	99.83 ±3.90	69.80 ±2.98	0.05	100.00 ±6.96	58.74 ±1.73	0.05
TLA (cm^2)	253.29 ±10.09	363.36 ±30.33	409.80 ±24.56	ns	713.98 ±72.84	537.67 ±54.47	ns	1112.82 ±76.29	693.48 ±47.94	0.05

SWC (soil water content); XS (xylem sap); L (leaf); ABA (abscisic acid); ASC (abaxial stomatal conductance); LWP (bulk leaf water potential); LTP (leaf turgor potential); LOP (leaf osmotic potential); LER (leaf expansion rate); TLA (total leaf area); WC (watered control); WS (water stress); P (level of significance) and ns (not significant at $p < 0.05$).

Table 6.2: Changes in SWC, XS ABA, L ABA, ASC, LWP, LTP, LOP and LER with time when poplar plants were grown in large soil volumes (*ca* 4000 cm^3) after withholding water from the soil. Two tailed paired student t-test. Values are means of four observations ± one standard error of mean.

Variables	**Time (days)**									
	1	3			5			8		
		WC	WS	P<	WC	WS	P<	WC	WS	P<
SWC ($g\ g^{-1}$)	0.35 ±0.01	0.35 ±0.01	0.27 ±0.003	0.01	0.33 ±0.01	0.19 ±0.01	0.001	0.32 ±0.01	0.13 ±0.002	0.001
XS ABA (μ mol m^{-3})	71.98 ±4.78	119.49 ±8.23	194.73 ±23.68	0.05	156.91 ±6.13	441.51 ±22.52	0.001	175.69 ±11.10	442.12 ±20.93	0.001
L ABA (ng g^{-1} dw)	17.85 ±0.50	17.78 ±0.88	22.58 ±2.45	ns	20.43 ±1.26	53.59 ±6.15	0.05	17.93 ±0.71	117.56 ±9.49	0.01
ASC (mm s^{-1})	11.00 ±0.34	14.08 ±0.51	9.67 ±0.43	0.05	13.77 ±0.65	7.61 ±0.34	0.001	14.62 ±0.49	4.86 ±0.32	0.001
LWP (- MPa)	0.80 ±0.08	0.85 ±0.05	0.90 ±0.08	ns	0.88 ±0.05	1.02 ±0..02	ns	0.86 ±0.05	1.11 ±0.05	0.05
LTP (MPa)	0.58 ±0.07	0.42 ±0.03	0.50 ±0.04	ns	0.40 ±0.02	0.38 ±0.03	ns	0.55 ±0.05	0.38 ±0.01	ns
LOP (-MPa)	1.38 ±0.01	1.26 ±0.04	1.39 ±0.07	ns	1.28 ±0.04	1.35 ±0.05	ns	1.40 ±0.02	1.49 ±0.05	ns
LER (% cont.m)	100.00 ±0.00	100.03 ±13.34	92.22 ±3.86	ns	100.01 ±7.78	85.88 ±7.17	0.05	100.00 ±3.17	77.55 ±6.05	0.05

SWC (soil water content); XS (xylem sap); L (leaf); ABA (abscisic acid); ASC (abaxial stomatal conductance); LWP (bulk leaf water potential); LTP (leaf turgor potential); LOP (leaf osmotic potential); LER (leaf expansion rate);WC (watered control); WS (water stress); P (level of significance) and ns (not significant at $p < 0.05$).

of control mean) of treated plants were also plotted against the corresponding bulk leaf water potential, bulk leaf ABA and xylem sap ABA concentration. The paired observations from the same individual were plotted against each other for stomatal conductance, but for leaf expansion rate the data were from different treated plants. Relationships was determined by linear regression analysis and the values of the coefficients of determination (r^2) are presented together with the graphs.

6.2.10 Data analysis

Means and standard errors of the means of each variable on each occasion were calculated and are presented in the form of tables. The paired data were tested by Students t-test using the STATVIEW package.

6.3 Results

6.3.1 Soil water content, xylem sap ABA and bulk leaf ABA

Withholding water from the soil induced a gradual decline in soil water content of the whole soil column with either bean (Table 6.1) or poplar (Table 6.2). However, in both cases the shallow soil layer dried faster than the deeper soil layer (data not shown). For bean on day 3 of the drying cycle, the reduction of soil water content of the shallow and deep soil layers was 33 and 12%, respectively (relative to the respective soil layers of well-watered soil): for poplar, the reductions were 29 and 20%, respectively. Thus, the roots in the shallow soil layer were subjected to a water deficit immediately after withholding water while the deeper parts of the root system should have been able to get water and maintain shoot water supply.

In association with the gradual decline of soil water content, xylem sap ABA and bulk leaf ABA concentration increased simultaneously in both bean and poplar plants. The initial (day 3) increase in bulk leaf ABA in unwatered plants was not statistically significant although the xylem sap ABA was significantly higher (2.26 and 1.63 times that of well-watered plants) in both bean (Table 6.1) and poplar (Table 6.2), respectively. On day 5 of the drying cycle, with a further decline of soil water content, the bulk leaf ABA concentration increased significantly ($p < 0.05$) in both species in association with increased xylem sap ABA.

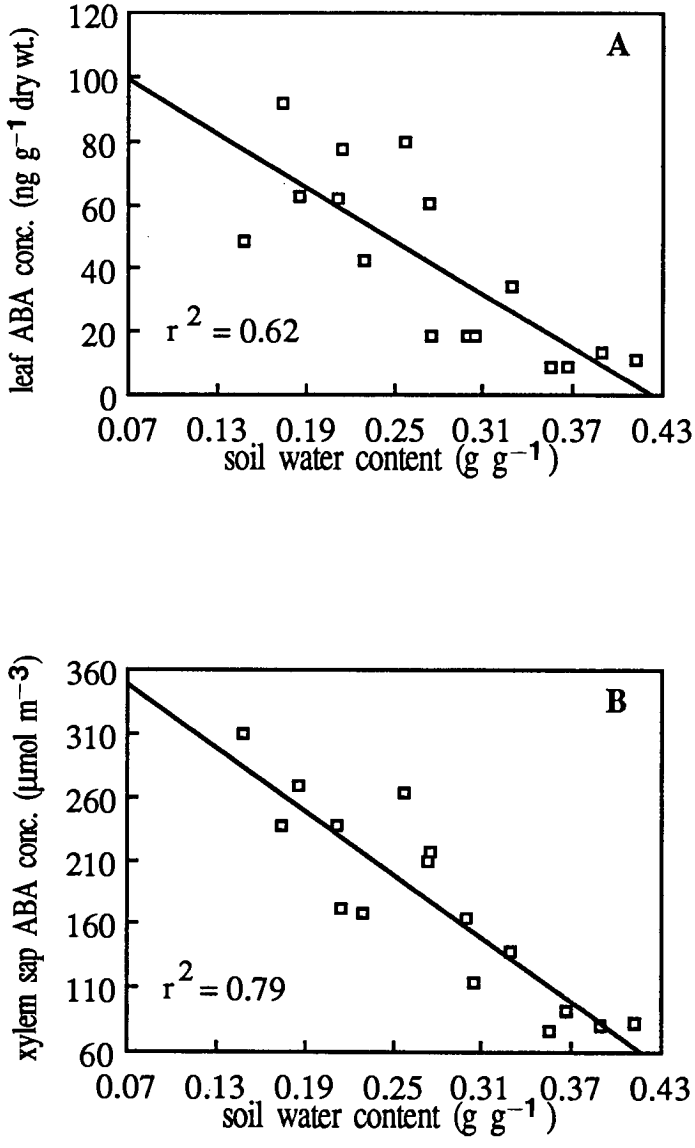

Figure 6.1: Relationship between soil water content and bulk leaf ABA (A) and xylem sap ABA (B) concentration of bean plants subjected to progressive soil drying. Points are results of individual plants on four successive occasions (d 1, 3, 5 and 8). Each point represents one plant. Measurements were made on four plants on each occasion. The regression line and the value of the coefficient of determination (r^2) are shown. Samples for ABA measurement were collected at 6-7 hours into the photoperiod.

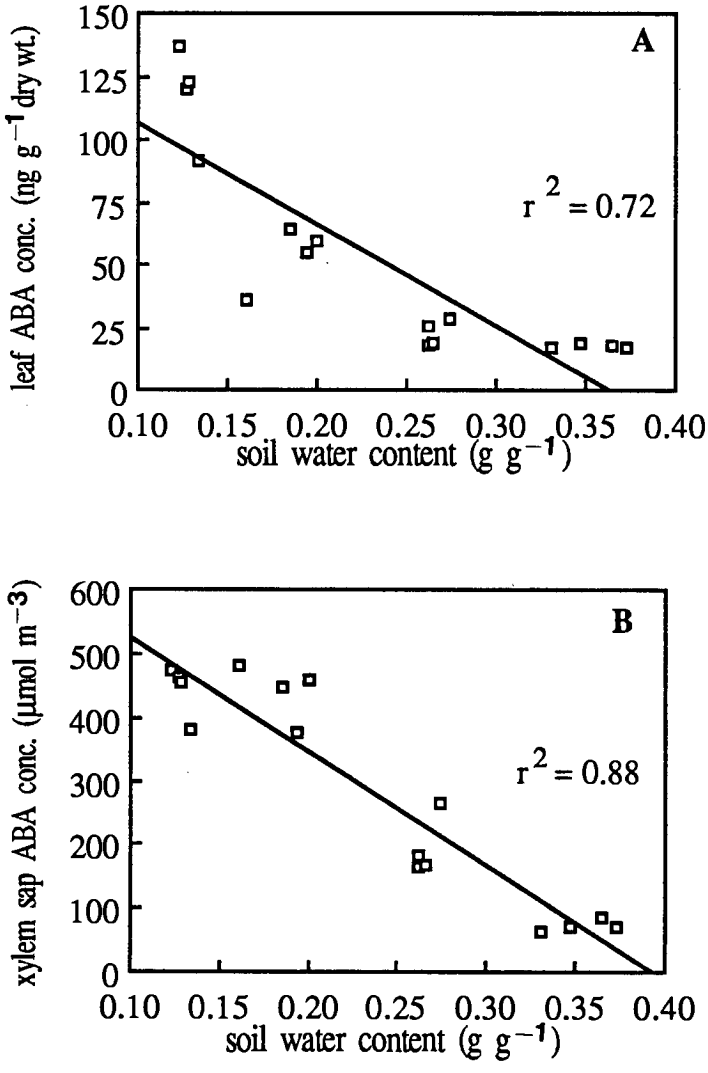

Figure 6.2: Relationship between soil water content and bulk leaf ABA (A) and xylem sap ABA (B) concentration of poplar plants subjected to progressive soil drying. Other particulars are as in Figure 6.1.

The bulk leaf ABA of unwatered bean plants on day 8 of the drying cycle was similar to that on day 5, while the xylem sap ABA was substantially higher (1.30 times) on day 8 when soil water content was 0.18 g g^{-1} (51% of the value in well-watered soil) (Table 6.1). In the case of unwatered poplar plants, the bulk leaf ABA on day 8 was significantly higher (2.19 times) than on day 5 but the xylem sap ABA remained unchanged. At this time, soil water content was 0.13 g g^{-1} (41% of that of well-watered soil) (Table 6.2), so that excessive soil dryness probably caused the transpiration stream from the dry roots to cease. Thus, during soil drying, the change in xylem sap ABA and bulk leaf ABA concentration may be independent of each other and can be related to changes in soil water status and later (in drier soil) to leaf water status.

The correlation between soil water content and xylem sap ABA was stronger (r^2 = 0.79 and 0.88) than the correlation with bulk leaf ABA (r^2 = 0.62 and 0.72) in both bean (Fig. 6.1) and poplar (Fig. 6.2) plants, respectively. Furthermore, the xylem sap ABA concentration, not the bulk leaf ABA, of well-watered plants of both species also gradually increased in association with the small decrease in soil water content of the well-watered soil over the experimental period (Table 6.1 and 6.2).

6.3.2 Leaf water relations

There were no significant differences between the leaf water relations of well-watered and unwatered plants of both bean (Table 6.1) and poplar (Table 6.2) until day 5 of the drying cycle. On day 8 of the drying cycle, both the turgor potential and bulk leaf water potential of unwatered plants of both species were substantially lower than in the well-watered plants.

6.3.3 Stomatal conductance

Stomatal conductance of the unwatered plants was reduced significantly in association with the decline in soil water content. The initial (day 3) reduction was associated with a significant increase in xylem sap ABA concentration in both bean (Table 6.1) and poplar (Table 6.2) whilst there was no change in bulk leaf ABA or leaf water relations.

On day 5 of the drying cycle, the reduction in stomatal conductance of both bean and poplar was associated with significant increases both in bulk leaf ABA and xylem sap ABA, yet without any significant reduction of leaf turgor potential and bulk leaf water

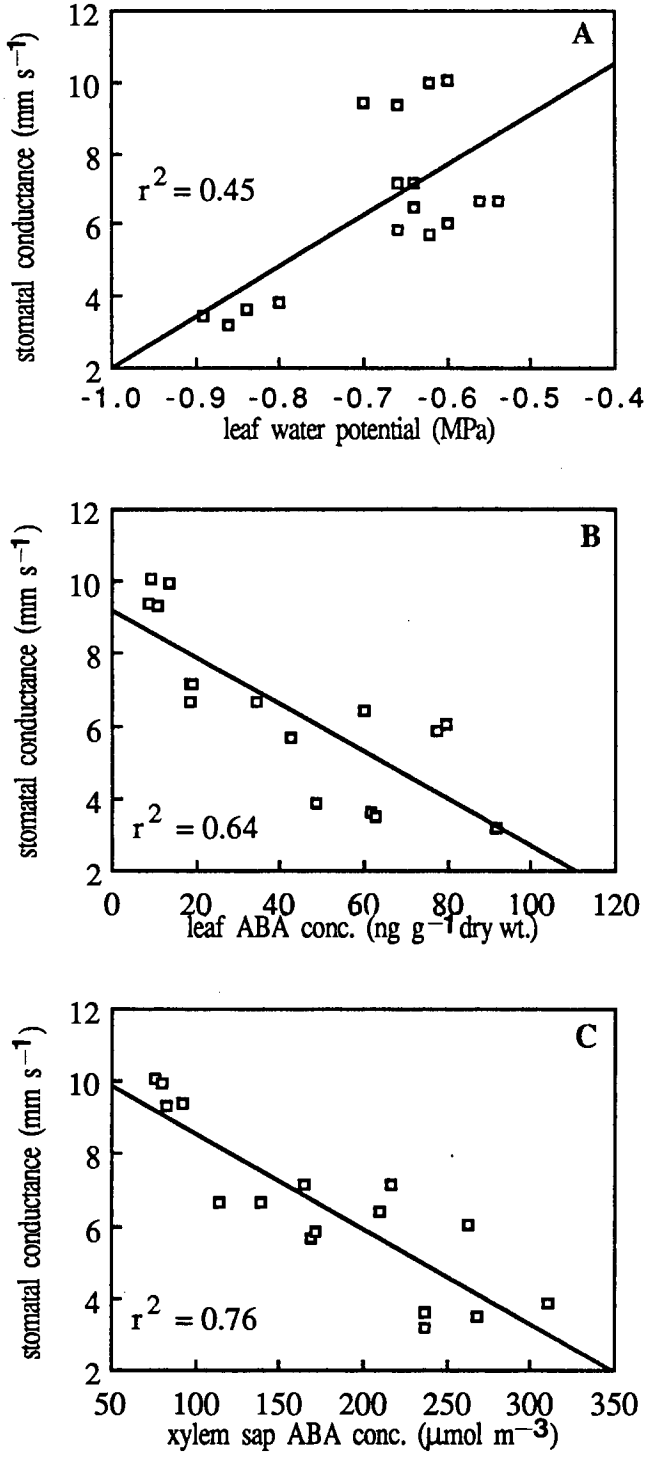

Figure 6.3: Relationship between stomatal conductance and bulk leaf water potential (A), bulk leaf ABA concentration (B) and xylem sap ABA concentration (C) of bean plants subjected to progressive soil drying. Other particulars are as in Figure 6.1.

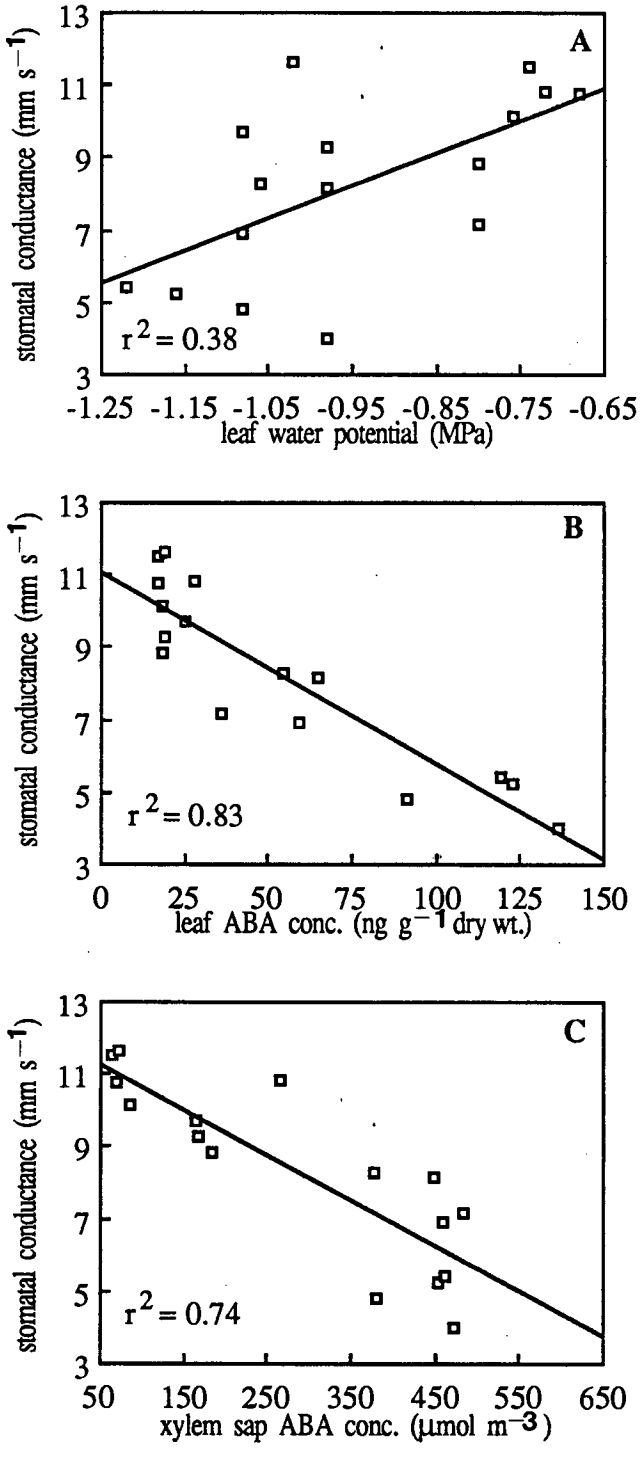

Figure 6.4: Relationship between stomatal conductance and bulk leaf water potential (A), bulk leaf ABA concentration (B) and xylem sap ABA concentration (C) of poplar plants subjected to progressive soil drying. Other particulars are as in Figure 6.1.

potential (Table 6.1 and 6.2). However, the reduction in stomatal conductance of both bean and poplar was highly significant ($p < 0.001$) and coupled with significantly lower bulk leaf water potential on day 8 when the soil was extremely dry (soil water content 0.18 and 0.13 g g^{-1}, respectively).

The correlation between stomatal conductance of unwatered bean plants and xylem sap ABA was rather stronger ($r^2 = 0.76$) than with bulk leaf ABA ($r^2 = 0.64$) (Fig. 6.3), whereas, in poplar the stomatal conductance was rather more strongly related to bulk leaf ABA ($r^2 = 0.83$) than to xylem sap ABA ($r^2 = 0.74$) (Fig. 6.4). In both species the relationship between stomatal conductance and bulk leaf water potential was significantly weaker (Fig. 6.3 and 6.4). Thus, both xylem sap ABA and bulk leaf ABA, are reasonably coupled in the regulation of stomatal conductance. However, the exact response possibly depends on species-specific redistribution of ABA in and out of the leaves.

6.3.4 Leaf expansion rate

The leaf expansion rate of unwatered plants (relative to the average leaf expansion rate of well-watered plants) was significantly reduced from day 5 of the drying cycle in both bean (Table 6.1) and poplar (Table 6.2). However, in the case of bean, a significant reduction of total leaf area was only observed on day 8 (Table 6.1). The correlation between leaf expansion rate and both xylem sap ABA and bulk leaf ABA of treated plants was rather stronger than the correlation with bulk leaf water potential in both bean (Fig. 6.5) and poplar (Fig. 6.6).

6.4 Discussion

The decline in stomatal conductance and leaf expansion of both bean (Table 6.1) and poplar (Table 6.2) plants rooted in large unwatered soil volumes (*ca* 4000 cm^3) before any decline in leaf water potential or turgor potential further confirms the effect of root signalling on the shoot, as reported earlier in this study (Chapter 5) and elsewhere (e.g. Blackman and Davies, 1985; Gollan *et al.*, 1986; Zhang *et al.*, 1987; Zhang and Davies, 1990a & b). The reduction in stomatal conductance and leaf expansion was associated with increased concentration of abscisic acid (ABA) in both xylem sap and leaves. Since, the increase in ABA (at least until day 5 of the drying cycle) occurred before any decline in leaf water potential or turgor potential, the increase is consistent

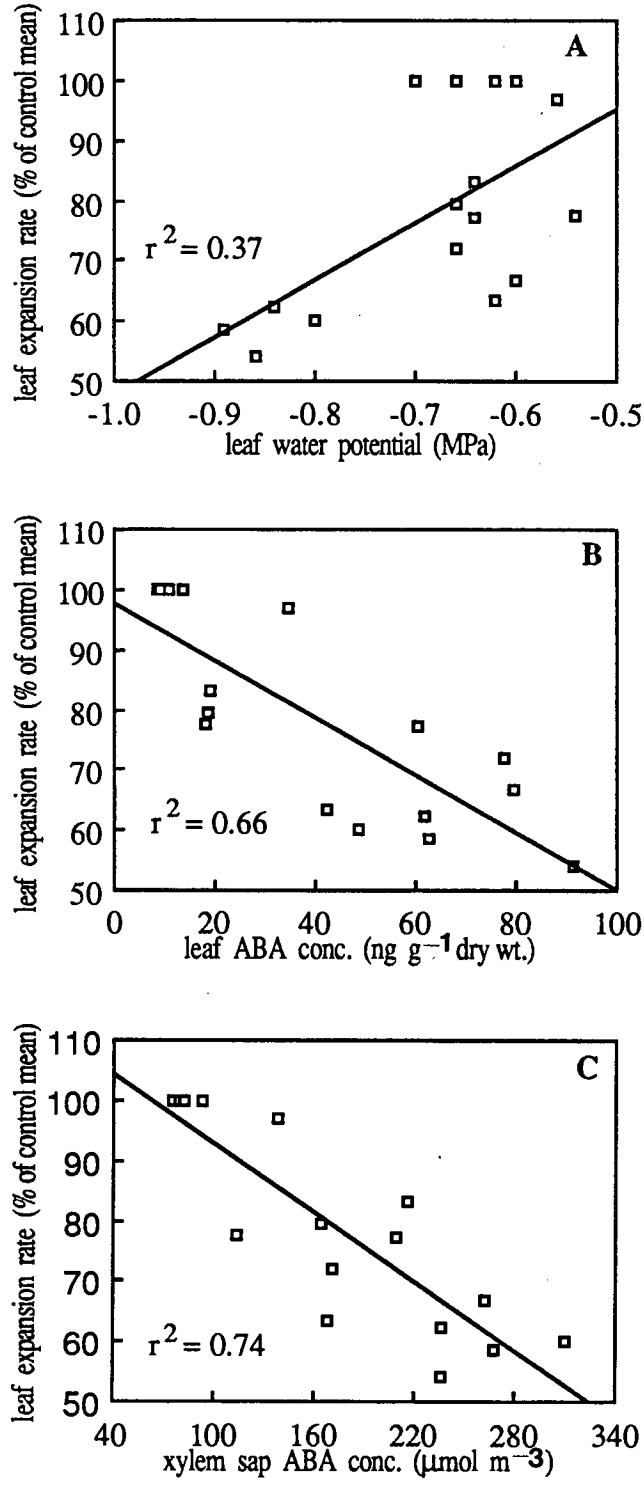

Figure 6.5: Relationship between leaf expansion rate and bulk leaf water potential (A), bulk leaf ABA concentration (B) and xylem sap ABA concentration (C) of bean plants subjected to progressive soil drying. The data of leaf expansion rate were from different treated plants. Other particulars are as in Figure 6.1.

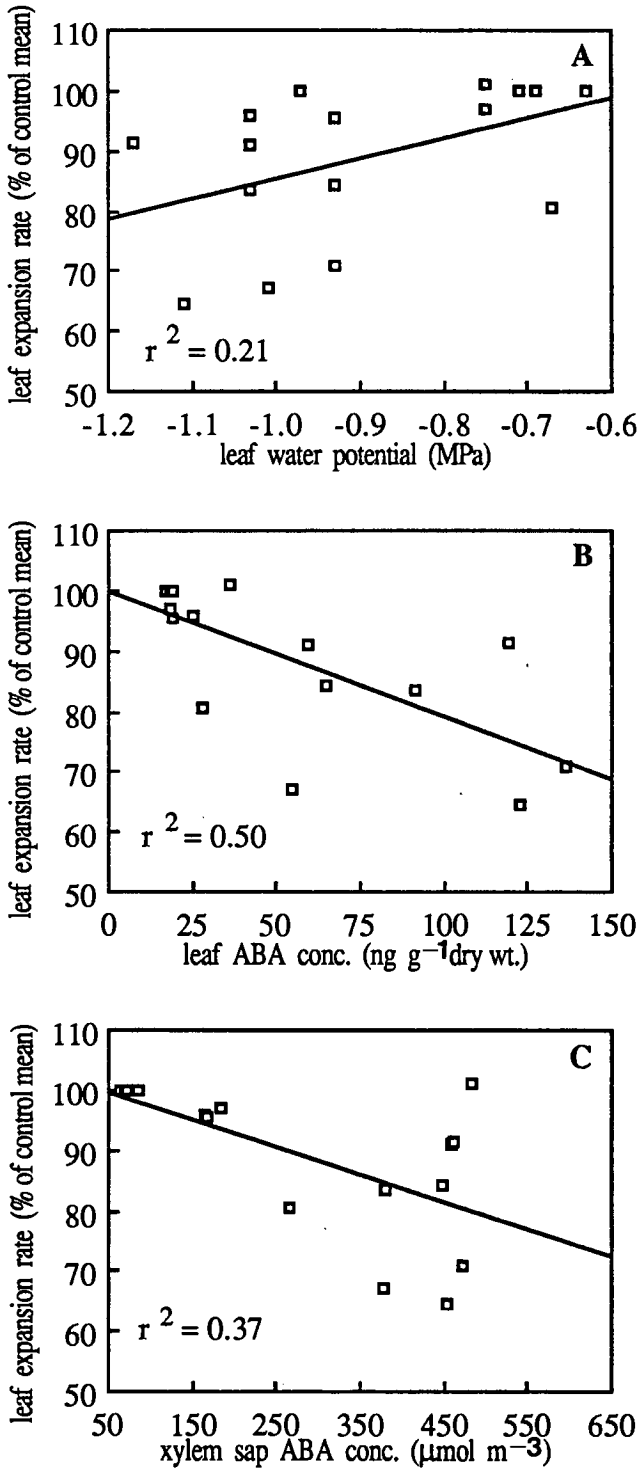

Figure 6.6: Relationship between leaf expansion rate and bulk leaf water potential (A), bulk leaf ABA concentration (B) and xylem sap ABA concentration (C) of poplar plants subjected to progressive soil drying. Other particulars are as in Figure 6.5 and 6.1.

with the hypothesis that ABA originates in the roots (Zhang *et al.*, 1987; Neales *et al.*, 1989; Zhang and Davies, 1989a & b).

The initial (day 3) increase in xylem sap ABA of unwatered plants of both bean (Table 6.1) and poplar (Table 6.2) before any change in bulk leaf ABA supports the idea that ABA is produced in the roots and is transported to the leaves in the transpiration stream. The stronger correlation between xylem sap ABA (rather than bulk leaf ABA) and soil water content of the whole soil column in paired observations of both bean (Fig. 6.1) and poplar (Fig. 6.2) also supports this argument. The close relationship between xylem sap ABA and pre-dawn leaf water potential in field grown maize (Tardieu *et al.*, 1992a) and *Prunus dulcis* (Wartinger *et al.*, 1990) may also be explained by a strong correlation between soil water and xylem sap ABA.

The subsequent increase in leaf ABA on day 5 of the drying cycle in both bean and poplar supports the hypothesis that the increased concentration of xylem sap ABA may eventually increase the ABA concentration in leaves (Zhang and Davies, 1989a). This was not the case in bean plants on day 8 (Table 6.1), perhaps because ABA accumulated in leaves is redistributed to the roots through the phloem stream (Wolf *et al.*, 1990) and may eventually be transported back to the shoot in the xylem stream, resulting in an additional increase in xylem sap ABA (Zhang and Davies, 1989b). On the other hand, in poplar the xylem sap ABA concentration on day 8 (relative to day 5) was not enhanced despite relatively more ABA in the leaves (Table 6.2). In this case, there seems to have been a very small xylem flow from the roots to the shoot because of excessive soil dryness (soil water content 0.13 g g^{-1}). The volume flow of water from roots to shoot through the xylem in the transpiration stream may account for changes in the ABA concentration of the xylem sap during soil drying and is likely to fall to vary low values in dry soil. This discrepancy between bean and poplar could explain the differential influence of bean and poplar on the soil drying process within the same period under similar environmental conditions, in addition to a possible species-specific redistribution of ABA within leaves.

Since xylem vessels are connected directly with the leaf apoplast, increased ABA in the xylem sap could simultaneously increase ABA concentration in the apoplast, bringing an ABA increase close to the stomatal guard cells and resulting in stomatal closure (Davies and Zhang, 1991). Hartung (1983) and Hornberg and Weiler (1984) have suggested that the action of ABA is on the outer surface of the guard cell plasmalemma. In this way, the stomata of both bean and poplar could respond to

xylem sap ABA *before* the accumulation of ABA in leaf cells, as reported in maize and sunflower plants (Zhang and Davies, 1989a & b).

The stronger relationship between stomatal conductance and xylem sap ABA (rather than bulk leaf ABA) of unwatered bean plants in paired observations (Fig. 6.3) also suggests higher stomatal *sensitivity* to xylem sap ABA, in agreement with the result of Tardieu *et al.* (1992b) in field grown maize plants. In contrast, in poplar plants the relationship between stomatal conductance and bulk leaf ABA concentration, which was relatively stronger (Fig. 6.4), may indicate a higher sensitivity of stomatal guard cells to bulk leaf ABA. Grantz and Meinzer (1990) also obsrved stomatal closure in association with an increase in bulk leaf ABA concentration of field and greenhouse grown sugarcane plants during soil dehydration.

In leaves, ABA is generally trapped in mesophyll chloroplasts and can also be released to the apoplast because of a decreasing pH gradient between the cytoplasm and apoplast, since the pH of xylem sap increases during soil drying (Gollan *et al.*, 1992). So, increased apoplastic ABA during soil drying may originate both from the leaf mesophyll and from the xylem sap. This ABA may be transported to other parts of the plant through the phloem stream before entering the xylem stream again. However, this may be unlikely in some situations because of a possible decrease in the pH gradient between the apoplast and the phloem vessels (Hartung, 1976), resulting in an accumulation of ABA in the apoplast of stressed leaves (Henson, 1984). During partial soil drying, accumulation of leaf epidermal ABA has also been observed, and explained on the basis of ABA redistribution in leaves (see Zhang *et al.*,1987). Zhang and Davies (1987) demonstrated increased ABA in leaf mesophyll in association with increased leaf epidermal ABA when roots of *C. communis* were loaded with ABA solution.

Since, bulk leaf samples (measured in this study) include apoplast, epidermis and mesophyll, the bulk leaf ABA concentration may also account for stomatal regulation by xylem sap, mesophyll or epidermal ABA. Thus at this stage the difference between bean and poplar plants may be attributed to differences in stomatal response to changed ABA concentration in both xylem sap and elsewhere in leaves. The greater variability of stomatal conductance in non-irrigated field-grown maize plants in the presence of increased xylem sap ABA (Tardieu *et al.*, 1992b) supports this argument, together with the weaker relationship between stomatal conductance and mid-day xylem sap ABA in that experiment.

The chemical signal from roots not only restricts stomatal conductance but has also been observed to reduce leaf expansion before any decline in leaf water status occurs (Masle and Passioura, 1987). This reduction was 30% in bean and 14% in poplar relative to well-watered plants on day 5 of the drying cycle and was associated with increased ABA concentration of both xylem sap and leaves (Table 6.1, 6.2), suggesting a negative influence of ABA on leaf growth. However, it is not clear how and where ABA acts in growth regulation. Since ABA acts on guard cell plasmalemma (Hartung, 1983; Hornberg and Weiler, 1984) in stomatal regulation, it is reasonable to suggest that the action of ABA is on the cell wall plasmalemma of expanding cells.

Cell expansion depends on cell wall loosening which may involve the activity of the plasmalemma. In the presence of ABA, the possible inhibition of proton secretion through the plasmalemma into the apoplast (see Zhang and Davies, 1990b) may eventually reduce cell wall extensibility (Van Volkenburgh and Davies, 1983) resulting in a reduction of leaf expansion. Zhang and Davies (1990b) in their experiments on maize and sunflower plants rooted in large soil volumes demonstrated a significant negative log-linear relationship between leaf expansion rate and xylem sap ABA but not with bulk leaf water potential. Similarly in our study, the relationship between leaf expansion rate and ABA concentration in xylem sap and leaves was stronger than with bulk leaf water potential in both bean (Fig. 6.5) and poplar (Fig. 6.6). The weaker relationship in poplar relative to bean may be related to the lower reduction of leaf expansion in poplar (22%) relative to bean (41%) within the same period (8 days) of soil drying under similar microclimatic conditions (Table 6.1 and 6.2).

However, traditionally, cell expansion is attributed to cell turgor (Boyer, 1968). This was not the case in the present study, where leaf expansion of unwatered plants apparently declined despite turgor maintenance (on day 5). Cell turgor potential is generally maintained either by higher water potential in cells of well-watered plants or by increased solute accumulation at lower water potential in the relatively smaller cells of unwatered plants. In the latter case the turgor driving force may not be effective in permitting cell expansion because of possible cell wall stiffening (Rhizopoulou and Davies, 1991) in association with lower cell water potentials. Therefore, it is the maintenance of turgor potential of growing cells in the presence of water rather than solutes which is important in maintaining cell expansion (Boyer and Nonami,1990). They suggested that the reduction in the water potential gradient between xylem and growing cells under conditions of water shortage in the root environment resulted in a

growing cells under conditions of water shortage in the root environment resulted in a decline in water potential of growing cells. Thus the reduction of leaf expansion growth under soil drying may be related to the coordinated influence of a change in cell wall properties and a lower water potential in growing cells.

The results presented here suggest that an apparent increase in xylem sap ABA concentration is the immediate response to soil drying before any change occurs in bulk leaf ABA, which might modify shoot functioning in the absence of a decline in turgor. However, the ABA concentration in the bulk leaf may also be increased later in as a result of the increased xylem sap ABA, without associated loss of turgor or reduction in bulk leaf water potential. The increased concentration of ABA in xylem sap can contribute subsequently to a substantial rise in bulk leaf ABA with the result that ABA in both the xylem sap and the bulk leaf may coordinate to modulate stomatal conductance and leaf expansion during soil drying.

CHAPTER 7

Conclusions and Recommendations

7.1 Introduction

Increase in productivity and sustainability from a particular unit of land is the prime objective of agroforestry, which involves an intimate association of different plant components, particularly trees and annual crops. In this association, interactions between component species in the exploration of environmental resources such as light, nutrients and water, is inevitable. Among these environmental resources, limitation of soil water is one of the most important plant stress variables in many parts of the world, not least in Bangladesh, because of the uncertainty and periodic variation of precipitation.

There is evidence of both competition (e.g. Thomas, 1984; Muthana, 1985; Campbell, 1989) and complementarity (e.g. Mathavan, 1985; Corack *et al.*, 1987) between component species in mixed cropping in relation to soil water supply. The growth and physiological behaviour of plants in an environment with a restricted supply of water is related to the competition and complementarity of the other components. Characterization of the growth and physiological behaviour of plant components in response to limited soil water is necessary for the selection of appropriate agroforestry species in an area subjected to water shortage. The attributes of a species identified in a short term experiment, particularly in a controlled environment, may be used to predict the performance of that species in the field under similar conditions. However, the accuracy of the prediction should be tested through small scale experiments in the field under similar conditions.

7.2 Limitation of soil water and root behaviour

Competition for soil moisture between trees and crop components in an agroforestry association raises the important issue of rooting depth, particularly during the establishment of tree seedlings. Deep penetration of root systems as a result of soil drying (Sharp and Davies, 1985) occurred in both bean and poplar plants when grown either alone or in mixed stands (Fig. 3.3), an adaptive mechanism in a drought prone

area allowing plants to explore deeper moist subsoil. However, in the present experiments soil moisture extraction by bean plants was restricted to the top 36 cm soil layer, whereas poplar explored the soil even below a depth of 50 cm (Fig. 3.2), indicating some complementarity between the two species. As a result, the relative yield total (RYT) exceeded 1.00 (Table 3.3B), suggesting that these two species do not compete for exactly the same below-ground resources (Remison and Snaydon, 1980), although root systems of both species were intermingled in the upper layers because of limited space inside the tube.

Rooting depth and root dry matter production, measured in this study, are not necessarily the best means of assessing root activity. Root length density is considered to be a better guide for determining the degree of exploitation of the soil, although it was not measured in this study. However, increase in root growth is manifested as an increase in both root dry weight and root length (Sharp and Davies, 1979). Competition for below-ground moisture is thus thought to be related to the development of the root systems of component species (Thomas, 1984). When soil water was limiting, the relative yield (RY) of the poplar root system in mixed stands was significantly smaller than that of the bean plants (Table 3.3A), resulting in significant suppression of the poplar yield (Table 3.4). This can be attributed to the greater competitive ability of the bean plants for soil water extraction because of their larger root system in mixed stands (Thomas, 1984). However with more space between component species during growth, such competition may be minimized. The results of the present work suggest that competition or complementarity between the component species of an agroforestry association for soil water depends clearly on the development of the root systems.

During soil drying, root system development varies not only between trees and herbaceous crops but also within both herbaceous (Molyneux and Davies, 1983) and tree species (Osonubi and Davies, 1981). This variation can be attributed to the capacity for solute accumulation and maintenance of turgor in the root tips during soil drying (Sharp and Davies, 1979), although these were not measured in this study. The conditions of soil drying may also change the development pattern of a root system even within the same species. For example, rapid soil drying induced a net increase in root growth of maize plants when grown in relatively small soil volumes (Sharp and Davies, 1979) in contrast to the root growth in slower drying, large soil volumes (Sharp and Davies, 1985). Thus, in a water shortage area root system development of the component species in an agroforestry association may be modified

relative to the root system development in a monoculture because of possible changes in the soil drying process.

Kummerow (1980) emphasized the importance of fine roots (< 1 mm diameter) in soil moisture extraction since fine roots comprise a substantial fraction of the root biomass. The significantly larger soil water depletion from the top soil layer (Fig. 3.1) can be attributed to the presence of a substantial amount of fine roots there, as well as to surface evaporation. In mixed stands this effect is intensified. Jonsson *et al.* (1988) observed a gradual reduction in fine roots with depth in tree-maize associations. Thus, in agroforestry shallow soil layers may dry faster resulting in dehydration of shallow roots long *before* the roots in the deeper soil layers and this can lead to the production of abscisic acid (ABA). Synthesis of ABA in dehydrating roots (Chapters 4 and 5) is well known (e.g. Zhang *et al.*, 1987). The increased ABA in roots enables enhanced root growth to occur (Saab *et al.*, 1990) as well as the regulation of shoot behaviour as a result of the transport of ABA from root to shoot in the transpiration stream (Zhang and Davies, 1987; Neales *et al.*, 1989) *before* any decline in shoot water status (Chapters 5 and 6).

7.3 Limitation of soil water and shoot behaviour

Leaf expansion and stomatal conductance of both bean and poplar plants declined during soil drying (Chapters 3, 4, 5 and 6). In accordance with the conventional view (see Hsiao, 1973), there were instances during the study when stomatal closure was accompanied by lower leaf water potentials in association with the decline in soil water status (Chapters 3 and 4). However, the correlation between stomatal conductance and mid-day leaf water potential was weaker than with pre-dawn leaf water potential (Fig. 3.10) suggesting a closer correlation with soil water status (Fig. 4.7 and 4.8) than with transpiration rate. This is in agreement with the earlier formulated hypothesis of a non-hydraulic influence of roots on shoot functioning during soil drying (Bates and Hall, 1981). The observed lower leaf water potentials referred to above can be attributed to the presence of an inadequate root system in the lower moist sub-soil (Fig. 4.1), in association with reduction in the water flow from roots in the upper dry soil to the shoot (see Zhang and Davies, 1989b). Thus the presence of a significant amount of roots in moist soil is important in maintaining an adequate shoot water supply in order to be able to identify non-hydraulic shoot characteristics responsive to early soil drying. In this study (Chapter, 5), it was found that during

soil drying plants with at least half of their root system in the moist part of the soil profile were able to maintain the same shoot water status as plants with all the root system in well-watered soil.

Generally, plants respond to reduction in the amount of available water in the soil by reducing their stomatal conductance and growth rate (Cowan, 1982). The observed reduction in stomatal conductance and leaf expansion *before* any perturbation of leaf water status in both bean and poplar plants during soil drying (Chapters 5 and 6) is an indication of their ability to "measure" the future availability of soil water (Turner *et al.*, 1985). Plants which are able to respond in this way can develop a pattern of efficient, long term utilization of water. Plants may differ in their sensitivity to soil drying. In this study bean plants appeared to be more sensitive than poplar as shown by their early reduction in stomatal conductance and leaf expansion and decline to low levels within a short period (Chapters 4 and 5). Similarly in a study of six birch species (Ranney *et al.*, 1991), river birch was considered to be the most sensitive species to mild water stress, as indicated by early decline in stomatal conductance and net photosynthesis. However, stomatal conductance was higher in the bean plants than in the poplar both in well-watered and unwatered conditions (Chapters 4 and 5) and this may have been the reasons for the higher photosynthetic rate of the bean plants. Henson *et al.* (1989) also reported higher photosynthetic rate of lupin than of wheat because of higher stomatal conductance in lupin.

Abscisic acid (ABA), synthesized in dehydrating roots in drying soil as a result of reduction in root tip turgor (Zhang and Davies, 1989a), was found to play a key role in the modulation of shoot behaviour through reduction of stomatal conductance and leaf growth (Chapters 5 and 6). The presence of a similar pattern of stomatal response to bulk leaf ABA in both bean (Fig. 5.3) and poplar (Fig. 5.4) during soil drying and dehydration of a part of the root system (Chapter 5) strongly supports an important role for ABA in stomatal regulation. However, increased xylem sap ABA is an immediate response to soil drying (Chapter 6) and can induce stomatal closure *before* any significant increase in bulk leaf ABA (Table 6.1 and 6.2), presumably because xylem vessels are directly connected to the leaf apoplast, through which ABA can be brought close to the stomatal guard cells.

The magnitude of the increase in xylem sap ABA required to induce stomatal closure varies from species to species (see Davies and Zhang, 1991). The increase in concentration of ABA in the xylem sap of unwatered bean and poplar plants for initial

stomatal closure was 2.26 and 1.63 times, respectively, that in well-watered plants (Table 6.1 and 6.2). Reduction in stomatal conductance within a narrow range of increase in xylem sap ABA (50-120 μmol m^{-3}) has also been reported in field grown almond trees (Wartinger *et al.*, 1990) and maize (Tardieu *et al.*, 1992a). These increases are smaller than was found in greenhouse-grown maize (Zhang and Davies, 1990a) and sunflower (Zhang and Davies, 1989b), where the magnitude of the increase in xylem sap ABA was *ca* 8 and *ca* 5 times, respectively, during mild soil drying. By contrast, increase in xylem sap ABA concentration of 50 fold in response to mild soil drying has been reported in wheat plants but there was no influence of such an increase in ABA on stomatal closure (Munns and King, 1988). Furthermore, Munns and King (1988) demonstrated the antitranspirant influence of xylem sap after the complete removal of ABA (using a radioimmunoaffinity column), a result that is difficult to reconcile with the present study and the work cited above. There is also evidence of initial stomatal closure in the absence of any ABA increase in the xylem sap of *Phaseolus vulgaris* subjected to soil drying (Trejo and Davies, 1991). Thus, stomatal sensitivity to increase in xylem sap ABA in relation to soil drying could be species-specific and this needs to be investigated further. None the less, the influence of environmental variation on stomatal sensitivity to ABA also can not be ruled out.

7.4 Role of root-shoot relations in agroforestry systems in dry regions

In agroforestry sustainable utilization of resources may be just as important as yield (Vose, 1981). Water use efficiency of a plant refers to the amount of water loss by transpiration per unit dry matter production. When plants respond earlier rather than later to soil drying by stomatal closure and reduction in transpiring area they are able to utilize available soil water more efficiently, because, early stomatal closure may reduce water loss without appreciable reduction in photosynthesis. Thus, in agroforestry competition between the component species for soil water can be minimized in a dry region because of lower transpiration demand. The use of component species with different water use efficiencies, that is species that require different amounts of water for similar amounts of dry matter production, may lead to the maintenance of sustainability of the agroforestry system in dry areas and this needs to be evaluated.

Nevertheless, significant proliferation of the root system throughout the deeper soil horizons during soil drying (Sharp and Davies, 1985) enables the whole soil profile to be explored. Root proliferation can occur as long as assimilates are available. Such assimilate allocation to the roots can be considered as an investment by the component species in an agroforestry system to compete effectively for below-ground resources. Translocation of sugar from shoot to roots of intact plants of *Phaseolus vulgaris* has been demonstrated when the root system was loaded with ABA (Karmoker *et al.*, 1979).

Since ABA is synthesized in dehydrating roots in drying soil, the root-shoot functioning of component species in an agroforestry system in a water shortage area might possibly be modulated by release of root-synthesized ABA in to the soil. Root cells, especially those from the root cap, may release ABA into the rhizosphere when the synthesis of root ABA by one component is relatively larger than by the other. Since the amount of ABA produced during water stress depends on the environment in which the plants are grown and the way in which the stress develops, such a mechanism needs to be investigated by growing plants separately and together.

7.5 The experimental approach and its evaluation

Plants growing individually in long soil columns (Chapter 3) are able to show their capacity for soil water exploration during soil drying as determined by root dry weight and root penetration. The responses determined without plant competition were not always similar to the results obtained with competition. However, although the dry weight of poplar roots was significantly lower in mixed stands (Fig. 3.2) the pattern of root penetration was similar whether grown alone or in mixed stands (Chapter 3). Problems may arise in separating the roots of two different species because of their overlapping root systems. Using a relatively large diameter soil column may provide enough space for root development and thereby minimize such limitations. Moreover, during soil drying the long soil column provides an upper shallow dry soil layer and a lower moist soil layer resulting in the root system of each plant being split horizontally in relation to soil water. Plants growing in large soil volumes in relatively shorter pots (Chapter 6) can also experience a similar situation. This enables the effect of partial root dehydration on shoot behaviour to be determined.

However, in these cases, a complete split-root situation may not always be maintained for a long period because of soil water extraction throughout the soil volume resulting from dense proliferation of the root system inside the limited pot space. Splitting the root system, both horizontally (see Section 4.2.1) and vertically (see Section 5.2.1), by artificial means is a good way of avoiding such limitations because of the complete separation of the root system of the same plant into two separate pots (Figs. 4.1 and 5.1). Horizontal splitting of the root system (Fig. 4.1), which is similar to the field situation, however, failed to maintain a sufficient water supply to the shoot, similar to that of control plants (Chapter 4), because of the difficulties of ensuring an adequate amount of roots in the lower moist soil during the drying of the upper soil column. Thus, vertical splitting of the root system into two pots (Fig. 5.1) is suggested as a simple and reliable method for the quick determination of the impact of root signals on shoot functioning since it allowed a continuous, ample water supply to the shoot from roots in the wet soil while half of the roots were in drying soil (Chapter 5).

With respect to leaf processes, continuous measurement of selected, young, growing, leaves (Chapters 4, 5 and 6) is a better option than measurement of recently fully expanded leaves (Chapter 3) as it avoids the subjectivity and possible sampling errors in the selection of recently fully expanded leaves on each occasion of measurement. Regarding leaf water relations, the measurement of osmotic potential of expressed sap using a vapour pressure osmometer (Chapters 4, 5 and 6) may sometimes lead to error in the determination of the calculated turgor potential because of possible variation in the dilution of the expressed leaf sap. Thus, the determination of leaf turgor potential by pressure volume analysis using a pressure chamber can be recommended, although it requires enough time for the series of measurements on each leaf. However, the measurement of bulk leaf water potential using a pressure chamber enables rapid assessment of the instantaneous total shoot water potential. The pressure chamber and transient diffusion porometer (used in the measurement of stomatal conductance) are simple, easy to handle in the field and reasonably cheap and so are acceptable technology for developing countries such as Bangladesh.

The method used for abscisic acid (ABA) analysis of plant samples in this study (see Appendix II) was simple and understandable although it requires a number of expensive instruments. Although the introduction of ABA analysis is currently not economically feasible in the developing world, knowledge of such technology provides a guide line during the identification of plant characteristics responsive to soil

water limitation. However, through the measurement of stomatal conductance and leaf water potential during soil drying the role of ABA on shoot functioning may be predicted, without the measurement of ABA. Thus, the measurement of root growth and leaf expansion, in association with the measurement of stomatal conductance and leaf water potential, can be considered as quick and useful methods in a screening programme for the selection of appropriate agroforestry species for a dry region of the developing world.

7.6 Implications of the study: relevance to agroforestry

The present study on root-shoot behaviour of an annual and a young tree was carried out as a model of a screening programme for the selection of appropriate agroforestry species. The characterization of root-shoot behaviour of annuals and young trees in relation to soil water supply in a relatively controlled environment provides a basis for predicting their performance in the field. For a region of limited soil water, no successful agroforestry management plan can be conceived without prior knowledge of the relationship between root-shoot performance of the individual components and soil water status. Assessment of characteristics related to this performance can be made by conducting small scale experiments which enable performance in the field to be predicted. Most characteristics of plants related to their response to soil water deficit identified in the literature have been derived from experiments with pot-grown plants. The growth and physiological responses of plants growing in pots can be reasonably related to their growth and physiological performance in the field, although not all annual crops and tree seedlings behave similarly. Possibly, soil drying in pots is relatively faster than in the field because of the dense root system that may develop in limited space within the pot.

Some annuals of high food value and some multipurpose tree species have been receiving considerable attention as potential agroforestry species in the developing world. With respect to Bangladesh, planting of tree-crop combinations in the homestead is a traditional land use system. The present agroforestry project and research has also been focussed on the aspect of maximizing productivity per unit land area without prior selection of appropriate species. In these circumstances, the study of root-shoot behaviour of agroforestry species by conducting short term experiments in partially controlled environments urgently needs to be carried out to assess the performance of possible component species in the field: important for a successful

agroforestry system. Prior to such a study a list of agroforestry species, on the basis of economic value and site characteristics, should be made. A number of non-timber economic crops, particularly herbs and shrubs (both wild and cultivated) of medicinal value should also be taken into account in the compilation of this list. The planting of such medicinal plants in mixed stands with trees and crops is also currently receiving considerable attention in the National Development Programme.

Thus, using the methods adopted during the present study a successful screening programme can be achieved for the selection of appropriate agroforestry species for a dry area. The method of growing plants in long soil columns (Chapter 3) is recommended since it enables root growth behaviour, an important determinant of competition between cohabiting species for below-ground resources (Singh *et al.*, 1989), to be determined realistically. Moreover, the response of stomatal conductance and leaf expansion to partial dehydration of the root system can also be determined since shallow roots are dehydrated during soil drying while deeper roots in moist soil maintain a sufficient shoot water supply (e.g. Zhang and Davies, 1989a).

From this study it is now possible to suggest that good understanding of the basic biology of trees and crops and their roles during soil drying would enable a successful agroforestry plantation to be established in a dry area. However, data on growth and physiology of component species are needed from small-scale field trials to ascertain whether the results observed in this type of study in pots in relatively controlled environments can be demonstrated in larger scale, agroforestry plantations. During such a field trial, the effects of plant density and shading of component species should also be assessed in association with below-ground interactions. Although water in the soil is the dominant variable regulating plant growth and development in dry areas, studies on nutritional effects are also needed to complete our understanding of below-ground interactions between the component species of an agroforestry system. Finally, it can be concluded that short term experiments are a necessary step in the attainment of a good understanding of the responsive characteristics of the component species in the establishment of any large scale agroforestry plantation. Thus the knowledge acquired on plant behaviour during this investigation provides a significant contribution to the future agroforestry programme of Bangladesh.

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APPENDIX I

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1. Analysis of variance for root dry weight (g)

Source	Degrees of Freedom (DF)	Sum of Squares (SS)	Mean Squares (MS)	Variance Ratio (*F*)	Probability (*P*)
Block	3	1.3647	0.4549	1.17	0.356
Water	2	0.2822	0.1411	0.36	0.703
Culture	1	5.1615	5.1615	13.22	0.002
Water*Culture	2	1.0032	0.5016	1.28	0.305
Error	15	5.8568	0.3905		
Total	23	13.6684			

2. Analysis of variance for stem dry weight (g)

Source	DF	S S	MS	*F*	*P*
Block	3	10.557	3.519	0.39	0.763
Water	2	207.218	103.609	11.43	0.001
Culture	1	91.377	91.377	10.08	0.006
Water*Culture	2	19.696	9.848	1.09	0.362
Eroor	15	135.927	9.062		
Total	23	464.776			

3. Analysis of variance for leaf dry weight (g)

Source	DF	S S	MS	*F*	*P*
Block	3	20.573	6.858	1.34	0.297
Water	2	10.670	5.335	1.05	0.376
Culture	1	55.693	55.693	10.92	0.005
Water*Culture	2	17.109	8.554	1.68	0.220
Error	15	76.487	5.099		
Total	23	180.532			

4. Analysis of variance for pod dry weight (g)

Source	DF	SS	MS	*F*	*P*
Block	3	281.20	93.73	1.48	0.260
Water	2	690.50	345.25	5.46	0.017
Culture	1	1291.11	1291.11	20.40	0.000
Water*Culture	2	317.88	158.94	2.51	0.115
Error	15	949.26	63.28		
Total	23	3529.94			

5. Analysis of variance for total dry weight (g)

Source	DF	SS	MS	*F*	*P*
Block	3	598.8	199.6	1.42	0.277
Water	2	1837.5	918.8	6.52	0.009
Culture	1	2850.4	2850.4	20.22	0.000
Water*Culture	2	692.1	346.0	2.46	0.120
Error	15	2114.2	140.9		
Total	23	8092.9			

6. Analysis of variance for total leaf area (dm^2)

Source	DF	SS	MS	*F*	*P*
Block	3	166.66	55.55	1.18	0.351
Water	2	383.46	191.73	4.07	0.039
Culture	1	307.45	307.45	6.52	0.022
Water*Culture	2	293.27	146.64	3.11	0.074
Error	15	706.99	47.13		
Total	23	1857.83			

7. Analysis of variance for root weight ratio (g g^{-1})

Source	DF	SS	MS	*F*	*P*
Block	3	0.0013	0.0004	2.28	0.121
Water	2	0.0010	0.0005	2.65	0.103
Culture	1	0.0000	0.0000	0.03	0.862
Water*Culture	2	0.0000	0.0000	0.23	0.794
Error	15	0.0028	0.0001		
Total	23	0.0053			

8. Analysis of variance for stem weight ratio (g g^{-1})

Source	DF	SS	MS	*F*	*P*
Block	3	0.0006	0.0002	0.64	0.602
Water	2	0.0121	0.0060	20.53	0.000
Culture	1	0.0006	0.0007	2.21	0.158
Water*Culture	2	0.0011	0.0006	1.97	0.174
Error	15	0.0044	0.0003		
Total	23	0.0189			

9. Analysis of variance for leaf weight ratio (g g^{-1})

Source	DF	SS	MS	*F*	*P*
Block	3	0.0008	0.0003	0.94	0.444
Water	2	0.0046	0.0023	8.76	0.003
Culture	1	0.0003	0.0003	0.97	0.340
Water*Culture	2	0.0001	0.0001	0.26	0.772
Error	15	0.0040	0.0003		
Total	23	0.0098			

10. Analysis of variance for pod weight ratio (g g^{-1})

Source	DF	SS	MS	*F*	*P*
Block	3	0.0016	0.0005	0.68	0.576
Water	2	0.0007	0.0004	0.46	0.637
Culture	1	0.0020	0.0020	2.60	0.128
Water*Culture	2	0.0006	0.0003	0.36	0.703
Error	15	0.0116	0.0008		
Total	23	0.0165			

11. Analysis of variance for leaf area ratio (dm^2 g^{-1})

Source	DF	SS	MS	*F*	*P*
Block	3	0.0030	0.0010	1.30	0.312
Water	2	0.0063	0.0031	4.15	0.037
Culture	1	0.0368	0.0368	48.22	0.000
Water*Culture	2	0.0080	0.0040	5.25	0.019
Error	15	0.0114	0.0008		
Total	23	0.0655			

12. Analysis of variance for specific leaf area (dm^2 g^{-1})

Source	DF	SS	MS	*F*	*P*
Block	3	0.5761	0.1920	2.93	0.068
Water	2	1.0341	0.5171	7.90	0.005
Culture	1	0.6176	0.6176	9.43	0.008
Water*Culture	2	0.0971	0.0486	0.74	0.493
Error	15	0.9823	0.0655		
Total	23	3.3072			

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13. Analysis of variance for root dry weight (g)

Source	DF	SS	MS	Variance *F*	*P*
Block	3	10.20	3.40	0.61	0.620
Water	2	790.21	395.11	70.66	0.000
Culture	1	327.01	327.01	58.48	0.000
Water*Culture	2	19.26	9.63	1.72	0.212
Error	15	83.88	5.59		
Total	23	1230.55			

14. Analysis of variance for stem dry weight (g)

Source	DF	SS	MS	*F*	*P*
Block	3	30.37	10.12	0.73	0.549
Water	2	683.71	341.86	24.72	0.000
Culture	1	721.94	721.94	52.19	0.000
Water*Culture	2	57.71	28.86	2.09	0.159
Eroor	15	207.48	13.83		
Total	23	1701.21			

15. Analysis of variance for leaf dry weight (g)

Source	DF	SS	MS	*F*	*P*
Block	3	35.73	11.91	0.57	0.644
Water	2	1467.79	733.89	35.08	0.000
Culture	1	1229.66	1229.66	58.78	0.000
Water*Culture	2	117.41	58.70	2.81	0.092
Error	15	313.78	20.92		
Total	23	3164.36			

16. Analysis of variance for total dry weight (g)

Source	DF	SS	MS	*F*	*P*
Block	3	98.30	32.80	0.34	0.797
Water	2	8517.00	4258.50	44.15	0.000
Culture	1	6400.00	6400.40	66.36	0.000
Water*Culture	2	514.20	257.10	2.67	0.102
Error	15	1446.80	96.50		
Total	23	16976.70			

17. Analysis of variance for total leaf area (dm^2)

Source	DF	SS	MS	*F*	*P*
Block	3	151.16	50.39	1.16	0.359
Water	2	3531.79	1765.89	40.53	0.000
Culture	1	2963.70	2963.70	68.02	0.000
Water*Culture	2	337.97	168.98	3.88	0.044
Error	15	653.56	43.57		
Total	23	7638.18			

18. Analysis of variance for root weight ratio ($g\ g^{-1}$)

Source	DF	SS	MS	*F*	*P*
Block	3	0.0081	0.0027	1.94	0.166
Water	2	0.0168	0.0084	6.06	0.012
Culture	1	0.0002	0.0002	0.17	0.683
Water*Culture	2	0.0030	0.0015	1.10	0.357
Error	15	0.0208	0.0013		
Total	23	0.0489			

19. Analysis of variance for stem weight ratio (g g^{-1})

Source	DF	SS	MS	*F*	*P*
Block	3	0.0049	0.0016	1.97	0.161
Water	2	0.0085	0.0042	5.13	0.020
Culture	1	0.0001	0.0001	0.09	0.764
Water*Culture	2	0.0005	0.0003	0.31	0.735
Error	15	0.0124	0.0008		
Total	23	0.0263			

20. Analysis of variance for leaf weight ratio (g g^{-1})

Source	DF	SS	MS	*F*	*P*
Block	3	0.0024	0.0008	0.97	0.435
Water	2	0.0014	0.0007	0.87	0.440
Culture	1	0.0000	0.0000	0.05	0.822
Water*Culture	2	0.0011	0.0005	0.67	0.527
Error	15	0.0123	0.0008		
Total	23	0.0171			

21. Analysis of variance for leaf area ratio (dm^2 g^{-1})

Source	DF	SS	MS	*F*	*P*
Block	3	0.0221	0.0074	2.46	0.103
Water	2	0.0756	0.0378	12.60	0.001
Culture	1	0.0341	0.0341	11.37	0.004
Water*Culture	2	0.0250	0.0125	4.17	0.036
Error	15	0.0450	0.0030		
Total	23	0.2020			

22. Analysis of variance for specific leaf area ($dm^2\ g^{-1}$)

Source	DF	S S	MS	*F*	*P*
Block	3	0.0938	0.0313	1.87	0.179
Water	2	0.2322	0.1161	6.93	0.007
Culture	1	0.2009	0.2009	12.00	0.003
Water*Culture	2	0.0715	0.0357	2.13	0.153
Error	15	0.2512	0.0168		
Total	23	0.8496			

RELATIVE YIELD

23. Analysis of variance for root dry weight (g)

Source	DF	S S	MS	*F*	*P*
Block	3	0.0249	0.0083	0.23	0.871
Water	2	0.0052	0.0026	0.07	0.930
Culture	1	0.5023	0.5023	14.24	0.002
Water*Culture	2	0.2177	0.1089	3.09	0.075
Error	15	0.5289	0.0353		
Total	23	1.2789			

24. Analysis of variance for stem dry weight (g)

Source	DF	S S	MS	*F*	*P*
Block	3	0.2495	0.0832	2.45	0.103
Water	2	0.0097	0.0048	0.14	0.868
Culture	1	0.5222	0.5222	15.40	0.001
Water*Culture	2	0.0417	0.0209	0.61	0.554
Eroor	15	0.5087	0.0339		
Total	23	1.3317			

25. Analysis of variance for leaf dry weight (g)

Source	DF	SS	MS	*F*	*P*
Block	3	0.1538	0.0513	1.50	0.255
Water	2	0.0468	0.0234	0.68	0.520
Culture	1	0.5290	0.5290	15.46	0.001
Water*Culture	2	0.1303	0.0651	1.90	0.183
Error	15	0.5134	0.0342		
Total	23	1.3733			

26. Analysis of variance for pod dry weight (g)

Source	DF	SS	MS	*F*	*P*
Block	3	0.2681	0.0894	3.53	0.088
Water	2	0.1142	0.0571	2.26	0.186
Error	6	0.1518	0.0253		
Total	11	0.5340			

27. Analysis of variance for total leaf area (dm^2)

Source	DF	SS	MS	*F*	*P*
Block	3	0.1808	0.0603	1.81	0.188
Water	2	0.1320	0.0660	1.98	0.172
Culture	1	0.6386	0.6386	19.19	0.001
Water*Culture	2	0.1188	0.0594	1.79	0.202
Error	15	0.4992	0.0333		
Total	23	1.5695			

RELATIVE YIELD TOTAL

28. Analysis of variance for root dry weight (g)

Source	DF	S S	MS	*F*	*P*
Block	3	0.0497	0.0166	0.23	0.869
Water	2	0.0103	0.0052	0.07	0.931
Error	6	0.4241	0.0707		
Total	11	0.4841			

29. Analysis of variance for stem dry weight (g)

Source	DF	S S	MS	*F*	*P*
Block	3	0.4990	0.1663	4.92	0.868
Water	2	0.0193	0.0097	0.29	0.761
Eroor	6	0.2030	0.0338		
Total	11	0.7213			

30. Analysis of variance for leaf dry weight (g)

Source	DF	S S	MS	*F*	*P*
Block	3	0.3077	0.1026	2.64	0.144
Water	2	0.0937	0.0468	1.21	0.363
Eroor	6	0.2328	0.0388		
Total	11	0.6341			

31. Analysis of variance for total leaf area (dm^2)

Source	DF	S S	MS	*F*	*P*
Block	3	0.3617	0.1206	2.81	0.130
Water	2	0.2639	0.1320	3.08	0.120
Error	6	0.2575	0.0429		
Total	11	0.8831			

COMPETITIVE PERFORMANCE

32. Analysis of variance for root dry weight (g)

Source	DF	S S	MS	*F*	*P*
Block	3	0.0511	0.0170	0.26	0.856
Water	2	0.0689	0.0345	0.52	0.605
Species	1	1.2885	1.2885	19.40	0.001
Water*Species	2	0.6210	0.3105	4.68	0.026
Error	15	0.9960	0.0664		
Total	23	3.0256			

33. Analysis of variance for stem dry weight (g)

Source	DF	S S	MS	*F*	*P*
Block	3	0.6164	0.2055	1.77	0.196
Water	2	0.0671	0.0336	0.29	0.753
Species	1	1.5010	1.5010	12.94	0.003
Water*Species	2	0.1104	0.0552	0.48	0.630
Error	15	1.7396	0.1160		
Total	23	4.0346			

34. Analysis of variance for leaf dry weight (g)

Source	DF	SS	MS	*F*	*P*
Block	3	0.1516	0.0505	0.43	0.733
Water	2	0.0835	0.0417	0.36	0.705
Species	1	1.3762	1.3762	11.78	0.004
Water*Species	2	0.3467	0.1733	1.48	0.258
Error	15	1.7529	0.1169		
Total	23	3.7108			

35. Analysis of variance for total leaf area (dm^2)

Source	DF	SS	MS	*F*	*P*
Block	3	0.3828	0.1276	1.38	0.287
Water	2	0.2220	0.1110	1.20	0.328
Species	1	1.3790	1.3790	14.92	0.002
Water*Species	2	0.1940	0.0970	1.05	0.375
Error	15	1.3865	0.0924		
Total	23	3.5644			

Appendix II

Radioimmunoassy (RIA)

RIA is based on the ability of animals to produce antibodies which can recognize and bind to specific foreign compounds in that animal. The antibodies (MAC62) used in this assay are specific for plant (+)-ABA. Labelled (radioactive) and unlabelled (free) ABA are mixed with antibodies. Both labelled and unlabelled ABA compete with each other during incubation to bind with the antibody. The antibodies with their bound components are separated and can be precipitated with ammonium sulphate. After removal of unbound material the amount of labelled ABA is measured as counts per minute (cpm). The amount of bound labelled ABA is inversely proportional to the amount of bound unlabeled ABA with which labelled ABA competes during the incubation.

II.1 Instrumentation for RIA

Eppendorf vials (1.5 cm^3) with separate press-on caps (Code nos. 72.908 and 65.697) were used to hold the RIA contents during assay. To support the vials 3 cm deep foam racks (Alpha Laboratories: Cat.no. AW 2625) were used. A micro-centrifuge (Eppendorf centrifuge Model 5413) which can hold forty 1.5 cm^3 eppendorf vials was used for spinning the RIA contents in eppendorf vials. A coulter mixer (Coulter Electronics Ltd.) was used for the purpose of over night extraction of samples. In order to shake the RIA contents in eppendorf vials, a whirlimixer (W-90, Laboratory FSA Supplies, England) was used. It was also used to resuspend and dissolve the pellet produced at the bottom of the vials during the assay. A repetitive pipette (BCL 8000) and pipettes P100, P1000 and P5000 (Pipetman P, Gilson, Medical Electronics SA, France) were used for accurate dispensing of the RIA solutions and sample extracts, ABA standard, scintillation liquid and water, respectively. For over-night extraction of samples and incubation of the RIA contents during assay a cold room, at 2 ^oC was used. A liquid scintillation counter (Model SL - 3000, Intertechnique) was used to count the specific activity (cpm) of the contents of each vial.

II.2 Reagents for RIA

Radio active ABA ([^{3}H] -ABA: ^{3}H-ABA (DL-cis, trans-[G-^{3}H]) with radioactive concentration 7.40 MBq cm^{-3} (Code TRV. 644, Batch 22) was procured from Amersham International plc, England.

Antibody (MAC62): The lyophilised antibody was kindly supplied by Dr. S.A. Quarrie, Cambridge Laboratory, JI Centre for Plant Science Research Ltd. UK.

Bovine γ-globulin (G-5009), bovine serum albumin (BSA, A-7030), polyvinyl pyrrolidone (PVP-40), (±) cistrans ABA (A-1012) and ammonium sulphate (A-5132) were procured from Sigma (Sigma Chemical Co. Poole, Dorset, UK).

Sodium di-hydrogen orthophosphate (NaH_2PO_4, Product -10245), di-sodium hydrogen orthophosphate (Na_2HPO_4, Product -10249) and sodium chloride (NaCl, product -10241) were procured from BDH (BDH Chemicals Ltd., England).

II.3 Stock solutions for RIA

Dilution of [^{3}H] -ABA: For the assay 20 mm^3 of original ^{3}H-ABA was added to 180 mm^3 of distilled water in a 1.5 cm^3 eppendorf vial and mixed thoroughly. The vial was covered with aluminium foil to avoid loss of radioactivity and stored in a freezer (-20 °C). With liquid scintillation counts per minute (cpm) of this stock were 85 000 to 95 000

Dilution of MAC62: The lyophilised stock was diluted to *ca* 950 mm^3 of distilled water (as recommended by the supplier). Aliquots of this solution, in *ca* 100 mm^3 lots were stored in a freezer (-20 °C) to use later in the assay.

Preparation of different concentrations of standard ABA: 5 mg of (±) cistrans ABA was put in to the bottom of a conical tube and mixed with 100 mm^3 of methanol (re-distilled). This mixture was sonicated briefly and 4.9 cm^3 of distilled water was added to make the concentration of ABA 1 mg cm^{-3}, named as stock-A. 100 mm^3 of stock-A was mixed with 9.9 cm^3 of distilled water so as to make a concentration of 1 µg/100 mm^3, named as stock-B. To make a concentration of 1ng mm^{-3}, named as stock-C, 100 mm^3 of stock-B was added to 0.9 cm^3 of distilled water. Five different

concentrations of standard ABA (0.25, 0.50, 1.00, 2.00 and 4.00 ng/50 mm^3) were prepared by mixing 5, 10, 20, 40 and 80 mm^3 of stock-C with 995, 990, 980, 960 and 920 mm^3 of distilled water, respectively in eppendorf vials and stored in the freezer (-20 °C) after covering with aluminium foil.

Scintillation cocktail: This was prepared by mixing the following ingredients, 3.3 g of 2.5-diphenylloxazole (PPO), 0.33 g of 1,4-di-2-(5-phenyloxazolyl)-benzen (PoPoP), 667 cm^3 of toluene and 333 cm^3 Triton X-100 and was kindly supplied from a laboratory of the Institute of Cell and Molecular Biology of the University of Edinburgh.

II.4 Fresh solutions for RIA

Preparation of phosphate-buffered saline (PBS): 0.79 g of NaH_2PO_4 and 0.7098 g of Na_2PO_4 were dissolved completely in separate 100 cm^3 of distilled water for each. The solution of Na_2HPO_4 was added slowly to the solution of NaH_2PO_4 until the pH reached 6.0 and then NaCl was added to that solution in the proportion of 0.58 g per 100 cm^3 of buffer. Some of the PBS was diluted 1:1 with distilled water to make 50% PBS. This was prepared on the day before the day of assay.

Buffer mixture for [3H] - ABA: The γ-globulin was dissolved in PBS at 5.0 mg cm^{-3} on the day of PBS preparation.

Buffer mixture for MAC62: BSA and PVP were dissolved in PBS at 5.0 mg cm^{-3} and 4.0 mg cm^{-3}, respectively, and was also prepared on the day of PBS preparation.

Ammonium sulphate saturated solution: Granules of ammonium sulphate were dissolved in distilled water by shaking in the proportion of 10 g per 12 cm^3 ensuring that plenty of residue remained at the bottom of the container. This was also prepared on the day of PBS preparation.

[^{3}H] -ABA solution: The stock solution of ^{3}H-ABA was mixed in γ-globulin buffer at 5.0 mm^3 cm^{-3} . This was prepared on the day of assay.

MAC62 solution: The stock solution of MAC62 was mixed with BSA/PVP buffer at 2.5 or 5.0 mm^3 cm^{-3}, as recommended by the supplier. This was also prepared on the day of assay.

II.5 Procedure for RIA

Methods of sample extraction:

Frozen samples of root and leaf were thawed and crushed into powder separately in a disposable plastic tube using a glass rod immediately after freezing in liquid nitrogen. The fresh weights (weight of thawed sample) of root and leaf samples were 100 mg and 150 mg respectively. Distilled water was mixed with the powdered mass of sample in the proportion of $1cm^3$/100 mg fresh weight. All the tubes were wrapped with aluminium foil and kept in a cold room (2-5 °C) on a coulter mixer (Coulter Electronics Ltd.) for over-night extraction (12-14 hours) by shaking. All the samples were centrifuged at 500 g for 5 min to clarify the extracts and the resulting supernatant was used directly in the assay.

Methods for RIA:

The caps of the eppendorf vials were removed and the vials were arranged on 3 cm deep foam racks. All the vials were labelled in order of maximum specific binding (Bmax), non-specific binding (Bmin), different concentrations of ABA standard (0.25, 0.50, 1.0, 2.0 and 4.0 ng/50 mm^3). There were three replicates of each standard and four of each sample.

The following amounts were added sequentially using a repetitive pipette (BCL 8000) and pipettes P100 (Pipetman P, Gilson, Medical Electronics SA, France): 200 mm^3 of 50% PBS to all assay vials, 50 mm^3 of water to vials for Bmax, 50 mm^3 of each standard ABA concentration to the calibration vials, and 50 mm^3 of sample to the sample vials. To the vials for Bmin 150 mm^3 of the lowest concentration ABA standard (0.25) was added. Subsequently 100 mm^3 of ^{3}H-ABA in γ-globulin solution and 100 mm^3 of MAC62 in BSA/PVP solutions were added to all vials, except that no MAC62 was added to the Bmin vials, so that the total content of each vial was 450 mm^3. Each vial was then capped quickly.

The contents of each vial were mixed thoroughly pressing the bottom of vials gently to the Whirlimixer and all the vials were incubated at 2 °C (in cold room) for *ca* 45 minute. At the end of incubation all the vials were spun briefly (1 min) using a micro-centrifuge (Eppendorf Model 5413) to remove liquid from the caps. 500 mm^3 of saturated ammonium sulphate solution was mixed into the contents of each vial and left at room temperature for 30 minute; later the spinning of all vials was carried out using the micro-centrifuge for 8 min at 8800 g. On completion of spinning the precipitated bound antibodies were pelleted at the bottom of the vials.

After removal of the caps of all vials the supernatants were discarded by turning the foam rack upside down. The caps were pressed face down onto paper towelling to remove any adhering moisture. In order to remove any residual supernatant adhering to the inside wall of the vials, the foam rack was tapped gently several times upside down onto paper towelling without dislodging the pellet. The pellet was washed later by resuspending in 1 cm^3 of 50% saturated ammonium sulphate solution, pressing the bottom of the vials gently onto the whirlimixer without any foaming. Again all the vials were spun using the micro-centrifuge for 10 min. Using the same methods stated in the previous step, the supernatants and any adhering moisture were removed completely. The pellet of each vial was successfully dissolved completely in 100 mm^3 of water by pressing the bottom of the vials on to the whirlimixer.

To this mixture 1.4 cm^3 of scintillation cocktail was added and mixed thoroughly. Each vial was put inside a 20 cm^3 empty scintillation vial and placed in a scintillation counter (Model SL-3000, Intertechnique) where it was counted once for 10 min. The amount of labelled (radioactive) ABA present in the pellet was measured as counts per minute (cpm), and is inversely proportional to the amount of unlabelled (free) ABA. The amount of unlabelled ABA concentration was estimated from the cpm (B) values, that were recorded in vials containing either standard ABA concentrations or samples, in the following way.

Calculation of ABA concentration: The values of cpm (B) were first transformed into LogitB which was calculated as the natural log of P/Q (ln P/Q), where P = (B-Bmin)/(Bmax-Bmin) and Q = 1-P. Here Bmin is the non-specific binding and B-Bmin and Bmax-Bmin are the corrected specific and maximum specific binding, respectively. A typical example of transformation of cpm to LogitB for five different concentration of standard ABA is given in the following table. The logitB for sample cpm was also calculated in the same way.

Table

ABA conc. (pg/vial)	B (cpm)	Bmin	Bmax	B-Bmin (*C*)	Bmax-Bmin (*D*)	*C* /*D* (*P*)	(1-*P*) (*Q*)	LogitB (ln *P* /*Q*)
125	1849.80	74.13	2377.45	1775.67	2303.32	0.771	0.229	1.214
250	1679.20	74.13	2377.45	1605.07	2303.32	0.697	0.303	0.833
500	1194.07	74.13	2377.45	1119.94	2303.32	0.486	0.514	-0.056
1000	868.80	74.13	2377.45	794.67	2303.32	0.345	0.655	-0.641
2000	668.80	74.13	2377.45	594.67	2303.32	0.258	0.742	-1.056

The values of LogitB (*Y*) for five different concentration of standard (+)-ABA (125, 250, 500, 1000 and 2000 pg/vial) were plotted against 0, 1, 2, 3, and 4 (*X* values) respectively. *X* values were calculated on a logarithmic scale with base two, because each standard ABA concentration increases by a factor of two, using the equation $X = \log_2$ [(+)-ABA concentration/125]. A linear regression equation between *X* and *Y* ($1.2616 - 0.6014X$; $R^2 = 98$) was derived (Fig. 1A). In this equation the slope *b* was negative and the constant *a* was positive. In order to calculate the estimated ABA concentration of each standard in each vial, the values of *a* and *b* were used in the following equation- $[2\ \{(\text{LogitB}-a)/b\}]$ 125. The values of *a* and *b*, derived from standard, were also used in the calculation of sample ABA using the same equation. In this example the estimated ABA for 125, 250, 500, 1000 and 2000 pg/vial standard were 132, 205, 571, 1120 and 1808, respectively. By plotting the cpm value of each standard ABA concentration against the corresponding standard ABA concentration a standard curve was also derived (Fig. 1B).

II.6 Validation of RIA

Although, application of RIA in the ABA analysis of aqueous extracts of leaves and roots as well as xylem sap of several species has been established (Quarrie et al., 1988; Zhang and Davies, 1990b; Trejo and Davies, 1991), the accuracy of the RIA was confirmed using the method of parallelism (Jones, 1987). Crude sample extracts of leaves and roots as well as xylem sap of both bean and poplar were assayed in the presence of different concentrations of added internal (+) - ABA standard in a range of

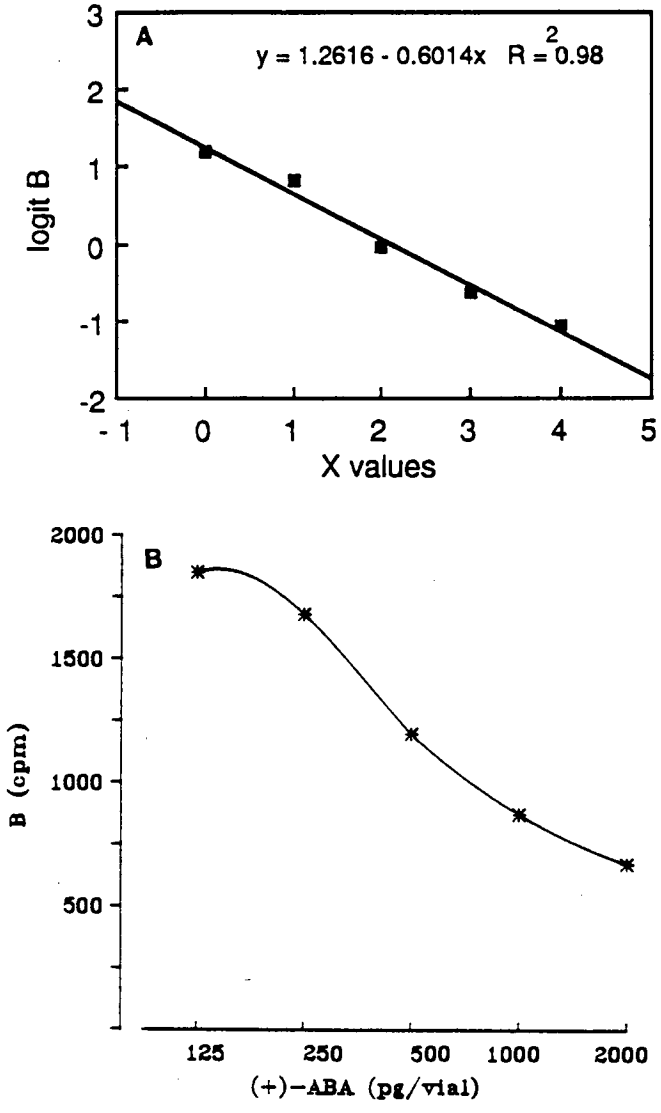

Figure 1: Linear regression (A) and standard Curve (B) for the RIA. Each point is the mean of three replicate RIA vials for each (+) - ABA concentration

dilutions (0 - 4). Both sample extracts and xylem sap were used as 100% and diluted to 50% and 25% with distilled water. Three replicate measurements for each concentration of both sample and standard were made. Within the limits of error, the regression lines were parallel when compared to the standard control line (Figs. 2 and 3). Thus there was no significant non-specific interference.

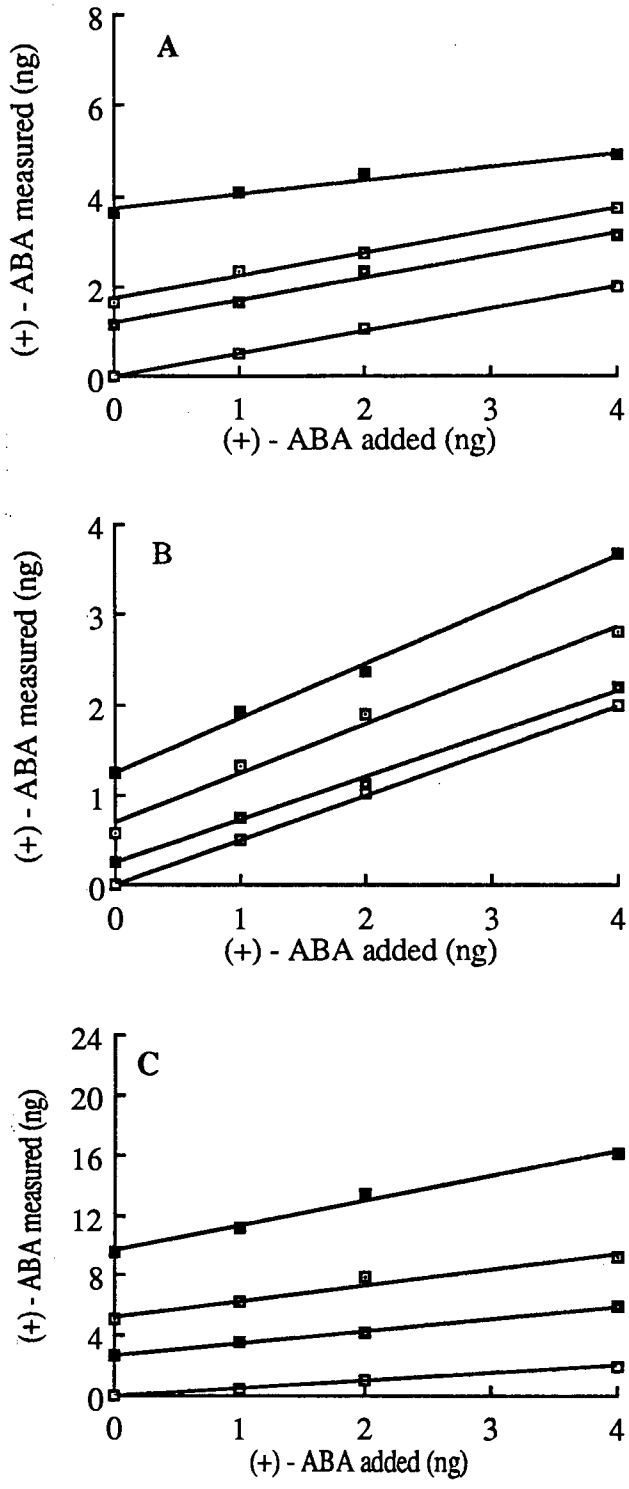

Figure 2: Dilution test for non-specific interference in the ABA radioimmunoassay of leaf extract (A), root extract (B) and xylem sap (C) of poplar in the presence of internal standards as a control □; samples not diluted ■; and diluted to 50% ⊡ and 25% ◘. Each point is the mean of three replicate RIA vials. The lines have been fitted by linear regression to the means.

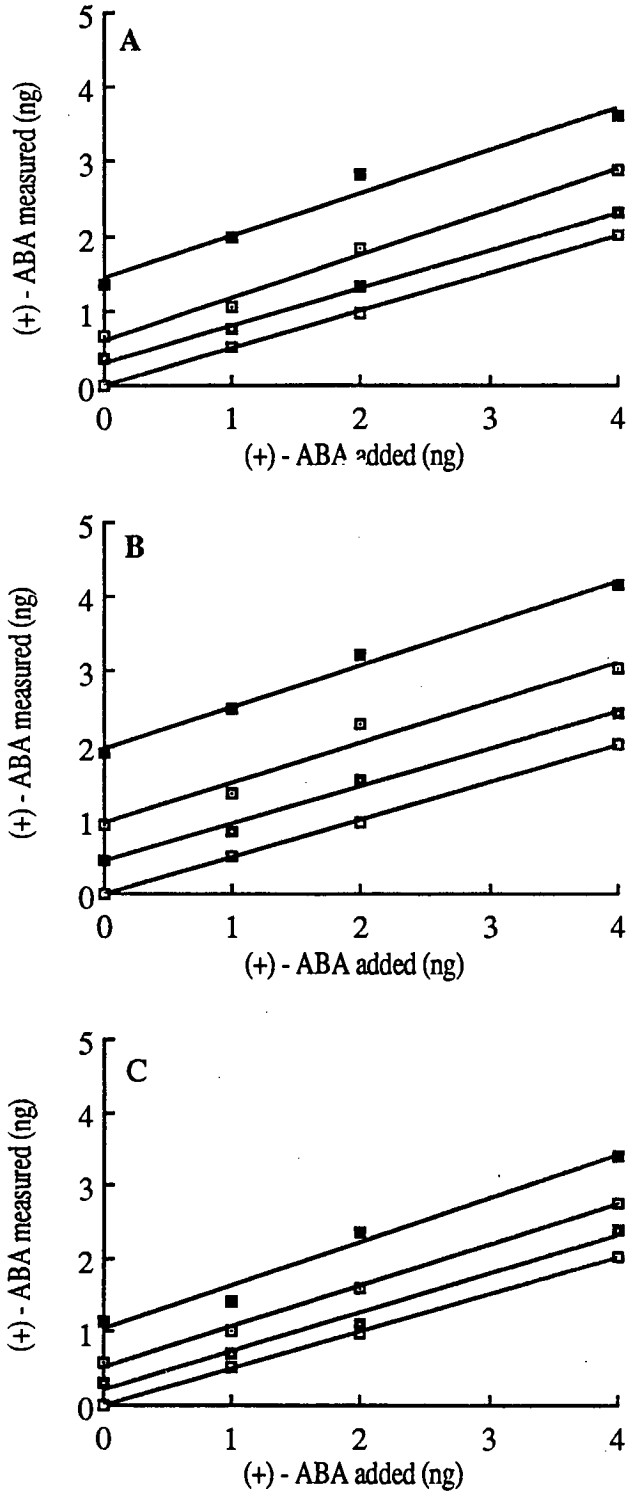

Figure 3: Dilution test for non-specific interference in the ABA radioimmunoassay of leaf extract (A), root extract (B) and xylem sap (C) of bean in the presence of internal standards as a control □; samples not diluted ■; and diluted to 50% ⊡ and 25% ◘. Each point is the mean of three replicate RIA vials. The lines have been fitted by linear regression to the means.